ACUTE AND CHRONIC HEPATITIS

CLINICAL AND EPIDEMIOLOGICAL ASPECTS OF ACUTE AND CHRONIC INFECTION WITH THE HEPATITIS B VIRUS WITH SPECIAL REFERENCE TO THE DELTA AGENT

by
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När dagen blir sen blir den late flitig.

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ABBREVIATIONS

HAV	hepatitis A virus
anti-HAV IgM	antibodies to the hepatitis A virus of IgM type
HBV	hepatitis B virus
HBsAg	hepatitis B surface antigen
anti-HBs	antibodies to the hepatitis B surface antigen
HBeAg	hepatitis B e antigen
anti-HBe	antibodies to the hepatitis B e antigen
HBcAg	hepatitis B core antigen
anti-HBc	antibodies to the hepatitis B core antigen
anti-HBc IgM	antibodies to the hepatitis B core antigen of type IgM
CAH	chronic aggressive (active) hepatitis
CPH	chronic persistent hepatitis

INTRODUCTION

Hepatitis A

The hepatitis A virus discovered in 1973 has recently been classified as enterovirus 72 (1). It contains a single-stranded RNA and only one known antigenic determinant giving rise to only one kind of antibody (anti-HAV). The infection is subclinical in many patients, particularly in children, and gives rise to lifelong immunity. It has never convincingly been found to cause chronic liver disease and a chronic carriership of the virus is not known.

The disease is transmitted by the faecal oral route and is endemic wherever the standard of public hygiene is low. In third world countries it is mainly a subclinical disease of childhood, in industrialized countries with their low degree of immunity it occurrs as epidemics caused by the ingestion of contaminated food or water or else is imported from endemic areas by travellers. Serologic surveys show that endemic transmission ceased in Scandinavia during the decades following world war II (2, 3). However, hepatitis A is not on the verge of disappearing in Scandinavia as suggested in a recent review (4).

In this present work we have analyzed the varying incidence of acute hepatitis A in our community during a decade and established risk groups.

Hepatitis B

The hepatitis B virus , HBV, also called the Dane particle, is a complex structure with several antigenic determinants all of which give rise to circulating antibodies. It has an outer protein shell, the hepatitis B surface antigen (HBsAg), the corresponding antibody is anti-HBs. There is extensive surplus synthesis of HBsAg in infected liver cells, which circulates in the blood as tubular and spheric particles unconnected with the viral core. These particles were first discovered by Blumberg (5) and called the Australia antigen.

The inner core of the virus contains the hepatitis B core antigen (HBcAg). It encloses the circular, to most part double-stranded virus DNA and a DNA polymerase. Also associated with HBV is the hepatitis B e antigen (HBeAg). It is a constituent of the HBcAg but can also be found free in serum as a soluble protein.

For a diagrammatic representation of the virus and its constituents se fig. 1.

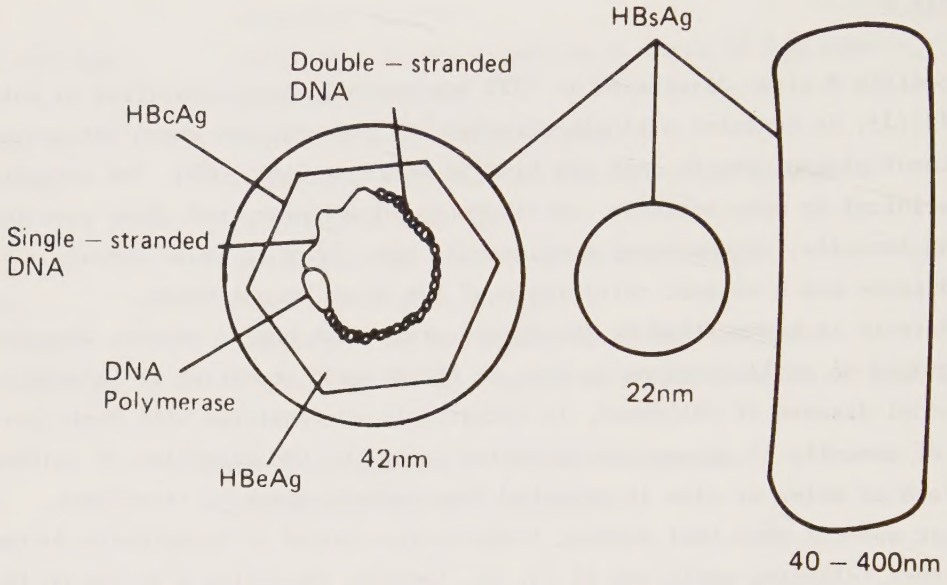

Fig. 1 Hepatitis B virus and its constituents

Hepatitis B is transmitted by blood-to-blood contact, whether through the skin as in the case of transfusions and injections or through mucous membranes during sexual contact. Five to 10 per cent of patients with acute hepatitis become chronic HBsAg carriers, a potentially oncogenic condition found in around 200 million persons around the world and in the vast majority resulting from infection at birth (South East Asia) or during childhood (Africa). Vaccines against hepatitis B have recently become available and mass eradication by vaccination campaigns in the newborn are being planned in high endemicity areas, i.e. areas with an HBsAg carrier rate in excess of 5-10 per cent.

We have here studied the yearly incidence of acute hepatitis B in our community during a decade in order to define risk groups. We have also made a long-term follow-up of patients with chronic hepatitis B comparing the characteristics of two major groups of HBsAg carriers.

HBeAg/anti-HBe

The HBeAg system was discovered by Swedish virologists (6) and has assumed great clinical importance. In chronic HBsAg carriers it is a sign of infectivity as shown by its association with DNA polymerase (7) and of liver damage (8, 9), in acute hepatitis it is useful as a marker of impending chronicity (8, 10, 11). Conversion from HBeAg to anti-HBe occurs in most HBsAg carriers, but there are definite differences in the rate of conversion in different types of patients with chronic hepatitis B (12, 13, 14) and also in healthy HBsAg carriers from different parts of the world (15, 16). In a long-term follow-up of two major groups of chronic HBsAg carriers (homosexual men and drug addicts) we have studied not only clinical and histological features but also defined the time of HBeAg-to-anti-HBe clearance and analyzed the differences between the two groups.

Hepatitis B virus DNA

Recent reports of infectivity even in HBsAg carriers positive for anti-HBe have caused concern and revealed the need of serological assays of greater specificity and sensitivity than HBeAg radioimmunoassay.

Since HBV will not grow in tissue culture other ways of demonstrating the complete virus must be used. By electron microscopy the Dane particles can be shown. Demonstration of the endogenous DNA polymerase is another possibility. Both these methods, however, are rather insensitive and laborious and require relatively large volumes of serum. Recently, molecular hybridization techniques to detect specific DNA sequences have been developed. By such methods HBV DNA can be detected both in the serum and the liver of patients with hepatitis.

In the first work on this subject (17) presence or absence of DNA in serum correlated well with presence or absence of HBeAg. In subsequent series (18 - 23) increasing numbers of anti-HBe positive chronic HBsAg carriers positive for viral DNA in their blood have been reported. However, the studies have all dealt with patients with chronic hepatitis. Our aim has been to examine hepatitis sera with molecular hybridization technique and to study the length of infectivity not only in chronic HBsAg carriers but also in patients with acute self-limiting hepatitis.

Delta agent infection

The discovery by Rizzetto in 1977 of the delta agent (24) opened up many new perspectives in the field of hepatitis research. The virus has a distinctive epidemiology, it has properties unique in virology and it is a serious cause of disease in many patients and a killer in some.

The delta agent was discovered during direct immunofluorescence studies of liver biopsies from HBsAg positive patients. It was noted that an antiserum against the hepatitis B core antigen as well as staining specimens in which core particles could be demonstrated by the electron microscope, also reacted with biopsies which did not contain core particles. Other reference antisera containing anti-HBc did not produce nuclear fluorescence, whereas some antisera without anti-HBc but positive for HBsAg did.

The new antigen-antibody system thus recognized was termed delta antigen and anti-delta (24).

After the time-consuming procedures of immunofluorescence had been replaced by radioimmunoassays for the detection of delta antigen as well as anti-delta large scale analyses of sera became possible and the dual nature of delta epidemiology became apparent. Delta infection is endemic in the general population in Southern Italy and in large parts of Africa and South America, whereas in Scandinavia it occurs almost exclusively in drug addicts (25).

The nature of the delta agent has been studied in experimental infection of chimpanzees (26) of three different categories. Sera from HBsAg positive patients with intrahepatic expression of delta agent were injected into 1) susceptible animals without markers of previous hepatitis B, 2) immune animals positive for anti-HBs and 3) animals which were chronic carriers of HBsAg.

In group 1) acute infection with HBV and the delta agent resulted ("delta coinfection"). In group 2) no infection occurred. In group 3) acute delta infection occurred ("delta superinfection") with a shorter incubation period than in group 1) and with a temporary lowering of the titer of HBsAg during the delta episode. Delta antigen could be detected in acute phase sera of delta hepatitis following pretreatment with detergent. In the serum the delta antigen was associated with particles precipitable by anti-HBs but not by anti-HBc or by anti-delta. Also contained in these particles was a very small single-stranded RNA. From these studies it appears that the delta infection is caused by a defective or incomplete virus which for its replication needs helper functions from HBV. In the serum the delta agent is present as a hybrid

particle whose interior contains the delta RNA and antigen and whose exterior is made up by HBsAg (28).

Extensive information is now available on many aspects of delta infection. In the present context two fields of study are of particular interest: epidemiology which was briefly mentioned above and clinical significance.

The clinical significance of delta infection in chronic HBsAg carriers has been studied by Italian and American workers who found severe liver damage in a large part of the patients (29, 30, 31).

Most investigators have found that acute hepatitis B with simultaneous delta infection has similar characteristics as acute delta negative hepatitis B. However, the delta agent has been found present in a considerable portion of patients with acute fulminant hepatitis.

For a recent overview of the present knowledge of delta infection by its discoverer, see 28.

In this work we have studied the epidemiology of delta infection in our community from 1970 and onwards and tried to define the clinical characteristics and the prognosis of delta infection.

Hepatitis non-A,non-B

As implied in the name the agent or agents responsible for hepatitis non-A, non-B have not been identified and the diagnosis can only be made by exclusion of hepatitis A, B and other causes of enzyme elevation. The disease has similarities with hepatitis B in that it is transmitted by blood and occurs in groups of patients exposed to blood such as intravenous addicts, patients receiving blood transfusions and haemophiliacs. A particular characteristic of this disease is its pronounced tendency to cause prolonged elevation of serum enzymes.

Incubation periods are intermediate between hepatitis A and B in some patients but very short (one to two weeks) in others which suggests at least two different viruses. In addition, a waterborne variant spread by the faecal-oral route has been described (32).

Our aim in studying hepatitis non-A,non-B was to establish its relative frequency as a cause of acute hepatitis in our community during a ten-year period and to define groups at risk of infection.

A recent comprehensive survey of hepatitis non-A,non-B is given by Dienstag (33).

AIMS OF THE STUDY

The aims of the present study were to investigate

1. the epidemiology of acute hepatitis A, B, and non-A,non-B in a well defined Swedish population over a period of more than 10 years,
2. the epidemiology, clinical presentation and prognosis in delta agent infection in the same population,
3. chronic HBV infection in a long-term follow-up of two major groups, intravenous drug addicts and homosexual men, with reference to clinical details, duration of infectivity as expressed by the length of HBeAg positivity and the degree of histologic liver damage and
4. the duration of viral replication and infectivity as expressed by presence of viral DNA detectable by molecular hybridization technique in patients with acute and chronic HBV infection.

MATERIAL AND METHODS

This investigation is based on all patients (approximately 2 000) with acute and chronic hepatitis seen at the General Hospital, Malmoe, Sweden, from 1970 onwards. This is the only hospital in the city which cares for such patients. Most of them are seen at the department of infectious diseases either directly or referred from other departments. The hospital as such is not a referral hospital and the patients are all inhabitants of the city with the exception of a low number of patients, usually drug addicts with hepatitis B seen at the city prison and sometimes referred from other parts of Sweden. The investigation, therefore, is based on an exclusively urban community with specific characteristics such as young adults addicted to drugs and sizeable communities of immigrants from less developed countries in Europe and overseas as well as of homosexual men, features of importance in a study of hepatitis.

The limited size of the city (pop. 250 000) has definite advantage in a study of epidemiology. The prospects of reaching all patients with a certain disease, of correct identification of sources of infection and of a more efficient follow-up are better than in a large metropolis.

The patient material examined here is expanding constantly and therefore is not the same in the various studies. The cases of acute hepatitis A, B, and non-A,non-B as of 1980 are enumerated on p. 15. Chronic HBsAg carriers available for study by the end of 1982 are listed on p.26.

Storing of sera started at the department of virology when methods for the detection of the Australia antigen (now HBsAg) were first introduced in 1970. Since then all sera have been saved and kept frozen.

Acute hepatitis was diagnosed on the basis of typical clinical signs and a more than ten-fold elevation of alanin-aminotransferase (ALT).

A chronic carriership of HBsAg was defined as HBs-antigenaemia for more than six months.

Liver biopsies were carried out with the Menghini technique. They were read by the same pathologist without prior access to clinical data. Diagnostic criteria were those of Bianchi et al (77).

HBsAg, anti-HBs, HBeAg and anti-HBe were determined by commercial radioimmunoassay kits (Abbott laboratories, North Chicago, Ill., USA).

Anti-HBc IgM was determined by solid-phase radioimmunoassay as described by Widell et al (78).

Delta antigen and anti-delta were determined by the original technique described by Rizzetto (27) with some modification (paper II).

HBV DNA was detected by a spot-hybridization technique described by Scotto et al (34). The HBV DNA probe was ^{32}P-labelled by nick translation.

RESULTS AND DISCUSSION

Acute hepatitis

Out of a total of 985 episodes of acute hepatitis seen during the period 1970-1979 311 (32 per cent) were diagnosed as hepatitis A, 494 (50 per cent) as hepatitis B and 168 (17 per cent) as hepatitis non-A,non-B. Twelve patients (1.2 per cent) had hepatitis A and B simultaneously.

Hepatitis A

Hepatitis A occurred in epidemics lasting between eight months and two years. This pattern was largely determined by the incidence in drug addicts who accounted for 58 per cent of all cases and for 70 per cent during the epidemics. Fifty-one (16 per cent) cases were connected with travelling, mainly to the Mediterranean area, 21 (6.5 per cent) resulted from family contact, three (0.9 per cent) occurred in homosexual men. In 55 (17 per cent) the source of infection was unknown.We may add to this that in the spring and early summer of 1984 a fourth epidemic is on its way. From the end of March through July approximately 30 cases of hepatitis A have occurred in drug addicts.

These figures are similar to those found in a study from Gothenburg comprising 473 patients seen 1974-1976 (35). They are different, however, from studies carried out in several other centers, notably in the absence or scarcity of hepatitis A in addicts in Melbourne (26), Copenhagen (37), Hannover (38) and Zurich (39). Some of these surveys may have been carried out between incidence maximums. This is a plausible explanation in regard to the study from Copenhagen (37), a city which is visited very frequently by drug addicts from our community. The study covered the period January 1977 to May 1978, which was a low incidence period in Malmöe practically without hepatitis A cases among addicts.

The Australian study, however, showed age specific prevalences of anti-HAV in several hundred addicts to be the same as in the average population. The conclusion must be that hepatitis A is prevalent among addicts in some communities but has not been introduced in others.

A similar situation prevails with regard to hepatitis A among homosexual men who account for less than one per cent of all cases in Malmoe and are absent in the Gothenburg study (35). However, in Stockholm an epidemic outbreak of hepatitis A occurred among homosexual men in 1979-80 and gave rise to 145

cases within 10 months (40).

Rhythmic variation of incidence is well known in many viral infections, a classical example being small-pox in the Indian subcontinent (41). The mechanism by which this pattern arises is presumably infection of a large part of susceptibles during an epidemic with consequent general immunity and disappearance of the infectious agent. In addicts the attack rates and consequent prevalence of immunity is likely to be especially high since prophylactic use of immunoglobulin is impractical. During the following years as new young persons are recruited into the drug habit a reservoir of susceptibles builds up and a new epidemic occurs when the agent is reintroduced. The quantitative requirements for a new outbreak to occur are not understood but the fairly equal length of the three epidemic intervals (three to four years) and the equal size of the three outbreaks (between 50 and 60 addicts) suggests a certain lawfulness. To test whether the model suggested is compatible with actual figures of immunity the prevalence of anti-HAV was analyzed in the sera of 234 addicts with acute hepatitis B seen during 1970-79 (42). The level of immunity to hepatitis A ranged from 7.7 per cent to 60 per cent, the lowest levels were found during the years preceeding the outbreaks and the highest immediately after. These figures, although not a proof, are compatible with the mechanism outlined above. It should be noted that in the second outbreak at least 39 per cent and in the third outbreak at least 54 per cent of the cases occurred in persons who had been intravenous addicts already during the previous outbreak. The attack rate in each epidemic therefore is less than maximal.

The use of cases of acute hepatitis B to study the prevalence of immunity to hepatitis A may introduce a bias similar to the one found in studies of homosexual men seeking medical assistance for venereal disease and taken to be representative of the homosexual community in general. A representative study of the addict population as such, however, is beyond our reach.

Although outside the scope of this investigation we would like to suggest that the epidemic curve of hepatitis A among addicts may be read to reflect the prevalence of addiction and that detailed analyses of heroin versus amphetamine users in the outbreaks might furnish information about the relative importance of these two drugs at various times and in various age groups.

Hepatitis B

Fivehundred-six cases of hepatitis B were noted, 236 (47 per cent) occurring in addicts. Family contact, homosexual contact and blood transfusion were responsible for 40 (7.9 per cent), 28 (5.5 per cent) and 13 cases (2.6 per cent) respectively. Thirty-two cases (6.3 per cent) occurred in hospital staff, 22 of these cases before 1975. In about one fourth of the cases no source of infection could be found. In figure 2 the original ten-year epidemic curve for acute hepatitis B has been redrawn to give the yearly incidence and the figures for four more years have been added. A definite pattern, less obvious previously, now comes out. Like in hepatitis A there are three peak incidence periods largely determined by the contribution of addicts. The first period covers 1971-73, the second 1977-78 and the third 1982. Two differences in this curve and the curve for hepatitis A are noted: whereas hepatitis A disappears among addicts during inter-epidemic periods, there is continued transmission of hepatitis B among addicts between the first two maximums. Secondly there is an over-all decrease in the incidence of hepatitis B notable both in the size and duration of hepatitis B epidemics among addicts and in the number of cases occurring without a known source of infection.

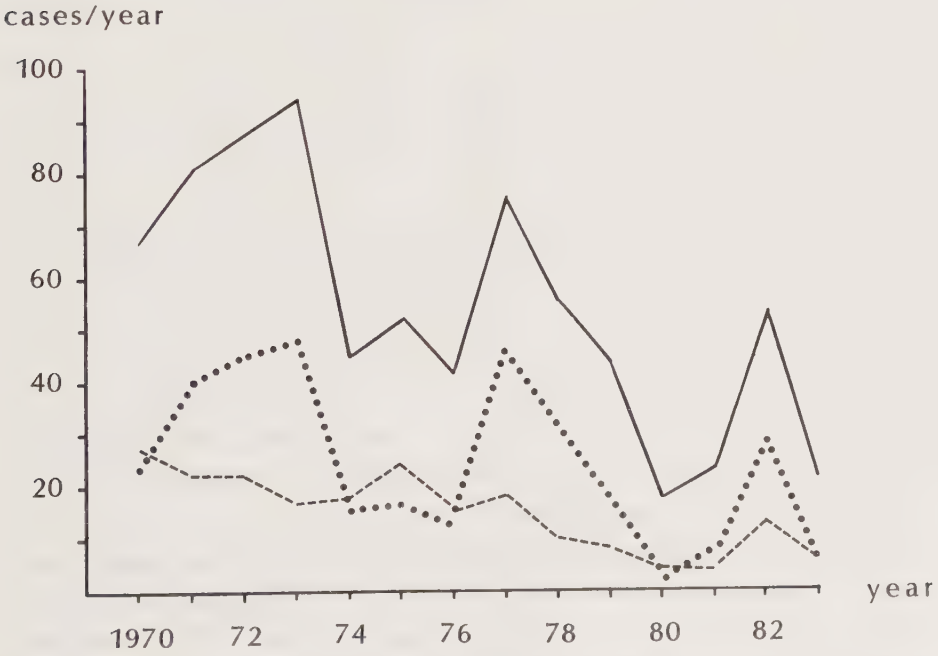

Fig. 2. Yearly incidence of acute hepatitis B showing total incidence (———), incidence in addicts (·····) and in patients with unknown source of infection (-----).

Possible explanations for the less dramatic epidemic curve in hepatitis B than in A are 1) the long and varying incubation period of hepatitis B which would of necessity blunt the epidemic peaks and 2) the role of chronic HBsAg carriers in the spread of infection. When judging the relative role of the second factor two important features should be born in mind. As shown in paper IV addict HBsAg carriers tend to loose their infectivity within a short time. After one and two years of follow-up 53.5 and 77 per cent, respectively, have lost infectivity as expressed by conversion to anti-HBe. The second important factor is the actual prevalence of chronic HBsAg carriers who can effect transmission of the disease.

Table I. Chronic HBsAg carriers registered yearly 1970 - 1983.

	Total	1970	71	72	73	74	75	76	77	78	79	80	81	82	83
Total	263	14	21	16	20	18	10	22	19	22	19	23	26	18	15
Addicts	89	6	11	10	10	10	3	8	8	7	5	2	4	2	3
Homosexual men	16					1		2	2	1	3	2	1	2	2
S.E.Asian refugees	31											7	12	10	2
Other known source of inf.	27	2	4	1		1	4	3	3	5	3		1		
Unknown source of infection	100	6	6	5	10	6	3	9	6	9	8	12	8	4	8

Table I shows that the yearly number of newly registered chronic carriers is on the same level throughout the period mainly because of the recent influx of HBsAg positive immigrants from South-East Asia. The number of new HBsAg carriers among addicts, however, has diminished considerably. Presumably their importance as a reservoir for endemic transmission among addicts has also dimished and this may in fact explain the shape of the curve in figure 2. During the seventies when the number of infectious HBsAg carriers was high the epidemic curve is blunted and the incidence of hepatitis B during inter-epidemic intervals only decreases to about one third of its value during high incidence periods. At the end of the period when most transmission is effected by patients with acute disease since there are few infectious chronic carriers available the inter-epidemic incidence drops to near zero and the picture as a whole comes to resemble that of hepatitis A. This effect thus is caused by the relative elimination of chronic HBsAg carriers as a source of infection.

On the whole, whereas the three outbreaks of hepatitis A seen during the seventies comprised similar number of patients, there is a definite decrease in the all-over incidence of hepatitis B. Total number of cases occurring among addicts during the three high-incidence periods 1971-73, 1977(-78) and 1982 were 143, 46 (76 for 1977-78) and 29, respectively. During the same period cases with unknown source of infection have decreased from approximately 25 per year 1970-72 to less than 10 per year 1980-81. The reason is not clear. Diminishing popularity of the drug habit is probably not an explanation since our experience with hepatitis A points to continuous recruiting of new addicts until the present time.

A striking reduction in the number of hospital staff infected with hepatitis B was recorded. Of a total of 32 cases 22 occurred before 1975. Since 1980 only one possible case is on record occurring in a laboratory technician in the department of pathology. This favourable development is commonly ascribed to greater awareness of the risks of infection, more efficient hygienic measures including colour-labelling of infectious blood samples and the use of prophylaxis with hyperimmune globulin. In our hospital groupwise vaccination of staff, apart from consideration of cost, is now considered undesirable. It is felt that a positive result obtained by a conscious effort on the part of each nurse and each laboratory technician in the long run is more reliable than a similar result achieved by vaccination which might easily induce an unwarranted sense of security followed by relaxation of vigilance. The next step might be reintroduction of the disease.

The factors operative in health care personnel to almost abolish hepatitis B as an occupational hazard would not explain the diminished incidence among addicts. The low incidence in addicts, however, in its turn might contribute to the low incidence in hospital staff.

Hepatitis non-A,non-B

Onehundred-sixty-eight cases of hepatitis non-A,non-B occurred, 103 were addicts (61 per cent) and 33 cases of unknown origin (20 per cent), the two largest groups in all three types of hepatitis. Blood transfusion and tourism abroad accounted for 13 and six cases respectively. Only one case occurred in a hospital worker.

The relative frequency of acute hepatitis non-A,non-B as compared to hepatitis A and B were 17 per cent, which is of the same order as found in Gothenburg (35), Copenhagen (37) and Stockholm (43).

The most important group were addicts who contributed 61 per cent of the

cases. An important finding is the near absence of clinically manifest hepatitis non-A,non-B in hospital staff (one case). Similar figures were found in the Gothenburg study (three per cent). There are a number of reports of needle stick-transmitted non-A,non-B in nurses (33). A case control study (57) of 238 patients in the United States with acute hepatitis non-A,non-B showed that six patients were employed in health care as against three among the controls. This was a significant difference (but only at the 0.05 level as compared with 0.01 for other risk factors).

In our study six cases (3.6 per cent) were ascribable to blood transfusion. This figure does not accurately reflect the incidence of post-transfusion non-A,non-B, a disease which occurs with a high proportion of asymptomatic cases (33). This was borne out by a subsequent prospective study of transfused patients carried out in our hospital in a prospective manner over a six week period in 1983. It was found on a basis of enzyme elevation that for each icteric patient there were 9 patients without symptoms or with unspecific symptoms not suggestive of hepatitis (Unpublished results).

Delta agent infection

Epidemiology

Onehundred-eighty-one chronic carriers of HBsAg and 599 patients with acute hepatitis occurring consecutively 1970-81 were analyzed for markers of delta infection.

Of the chronic HBsAg carriers 80 were drug addicts, 41 of whom were found positive for anti-delta. Of the remainder only three had anti-delta, a haemophiliac and two immigrants (from Turkey and from West-Germany). All sera from the period 1970-72 were negative for delta markers and thus the time of introduction of the delta agent could be established as the year 1973, when two brothers, both drug addicts, were infected. Since then the prevalence of delta has increased, in the three year period 1979 to 1981 72 per cent of all addicted HBsAg carriers available for study were positive for markers of delta infection.

Among patients with acute hepatitis B the first case of delta infection also occurred in 1973. Interestingly, this was a non-addict, a tourist who underwent emergency surgery during his holidays in Spain and received several blood transfusions. The first cases of simultaneous HBV and delta infection in addicts were noted in 1975. The yearly incidence of delta infection in addicts

with acute hepatitis B has remained around 40 per cent since then. In all, 50 addicts and five non-addicts were found positive 1973-81, one was the tourist already referred to, two had sexual contact and two non-sexual contact with addicts.

It should be emphasized that markers of delta infection were absent in all homosexual men analyzed (15 HBsAg carriers, 36 cases of acute hepatitis).

The high prevalence of delta infection in Scandinavian drug addicts was first noted in a European multi-center study (44) followed by our extensive study (paper III). Subsequently a large out-break occurred among heroin users in Dublin (45). Delta infections have also been described in other groups of patients with high prevalences of hepatitis B such as haemophiliacs (46) and to a lesser extent in male homosexuals in the United States (47).

In our community delta infection was introduced in 1973 as shown by its absence in earlier sera and its subsequent increase in chronic HBsAg-carrying addicts. The origin of delta infection i Malmoe is not known. Interestingly the first case of delta infection in acute hepatitis B has a known source of infection, the patient received blood transfusions during a visit to Spain. It is, however, highly improbable that this patient should be responsible for the subsequent spread of delta infection i Malmoe since he had no association with addiction and was the only example of simultaneous acute HBV and delta infection in a non-addict. The three chronic HBsAg carriers who were non-addicts were either middle-aged foreigners (two) or had probably been infected by imported plasma products (a haemophiliac).

Therefore, whether the source of the subsequent wave of delta infection was a foreign immigrant (seven of 41 delta infected chronic HBsAg carriers were non-Scandinavians) or a Swede visiting epidemic areas remains unknown.

In a study of Norwegian drug addicts it has been shown that delta infection was probably introduced into Norway in 1975 (48).

Delta infection has occurred among homosexual men in the United States (47). Its absence so far among homosexual men in our city and in Copenhagen (49) is, with a view to the serious prognosis of chronic delta infection dealt with below, a reassuring finding. In the case of Malmoe it may be explained by the virtual absence of overlapping between the two groups which is also illustrated by the predominance of sub-type ay among addicts as against 100 per cent prevalence of sub-type ad among homosexual men (Unpublished results).

Clinical findings

In 26 chronic HBsAg carriers in whom the time of conversion to anti-delta could

be established by serial analyses it was found that the most common clinical manifestation of delta infection was an episode of acute hepatitis with elevation of serum enzymes lasting for up to two months. In eight this episode lasted for up to one year. In one there was acute fulminant fatal hepatitis. There was biphasic elevation of enzymes in three of these patients. Enzymes were elevated by a mean factor of 46 (range 10-230), serum bilirubin by a factor of 10 (range 0.5-30) in 17 patients but normal in four and not measured in five. In 18 patients intermittent depression of HBsAg titers during the acute delta episode was noted. In three more patients the depression became permanent resulting in loss of HBsAg and development of anti-HBs. In two patients the HBsAg titers were unchanged.

In 25 carriers in whom the time relationship between the onset of both hepatitis B and delta infection was known wide variations were seen ranging from a simultaneous onset to a time lag of seven years. Eight patients were positive for HBeAg, 18 for anti-HBe, when delta infection occurred. A spontaneous clearance of HBsAg occurred in 14 drug addicts, 9 of whom were delta positive, five delta negative. As already mentioned the HBsAg clearance was related in time to acute delta infection in three of these.

In 57 of the patients with acute simultaneous HBV and delta infection 40 had classical acute hepatitis without distinctive clinical and laboratory features. In eight (14 per cent) a biphasic elevation of bilirubin and serum enzymes was noted.

Delta infection is either coinfection in which acute HBV and delta infection occur simultaneously or superinfection, i.e. acute delta infection superimposed on an already established chronic HBsAg carriership.

This difference has important implications for the subsequent course of the disease but also creates different clinical situations and may influence the clinical presentation.

In our series of delta coinfection it was often impossible to distinguish acute hepatitis B with and without delta infection on clinical and laboratory grounds. Fourteen per cent of these patients had a biphasic pattern of infection with two peaks of bilirubin and enzyme elevation which, however, is not pathognomonic of delta infection since it was seen in 15 of 535 cases of delta negative acute hepatitis B. However, the difference is highly significant ($p < .001$). In superinfection this was noted in three patients only. It was seen by Rizzetto et al in transmission experiments (26), namely in one of the two chimpanzees that were already chronic HBsAg carriers when infected with the

delta agent, and in patients by Raimondo et al (44) and by Caredda (50). Raimondo et al suggested that it was due to double infection with sequential expression of HBV and the delta agent. In those instances where biphasic delta hepatitis is seen in chronic HBsAg carriers it must have another explanation since in such patients the HBV infection as expressed by titers of HBsAg is depressed.

In cases of superinfection the diagnosis may be suggested by the unexpected occurrence of acute hepatitis in an otherwise stable HBsAg carrier. However, in such a situation the possibilities of confusion with other types of hepatitis are legio, the most important being hepatitis caused by alcohol, drugs, HAV, and the agent(s) of hepatitis non-A,non-B. When a hitherto unrecognized HBsAg carrier presents for the first time during acute delta positive hepatitis he can easily be misinterpreted as a simultaneous onset of HBV and delta infections leading to chronic carriership of HBsAg (51). Three such instances are recorded in our series, all occurred before the discovery of the delta agent and were misdiagnosed accordingly. The absence of anti-HBc IgM of high titer in the initial serum sample will lead to the correct diagnosis of delta superinfection in an already established HBsAg carrier. Occasionally this may be suspected by finding anti-HBe in the first serum sample, a sign that the HBs antigenaemia has already been present for some time (55).

Prognosis and histology

The histological changes in addicts who were chronic HBsAg carriers were evaluated by biopsy in 27 patients of whom 14 were positive and 13 negative for delta markers. In the delta positive group eight had chronic aggressive hepatitis (with cirrhosis in one), four had chronic persistent hepatitis and one had unspecific changes. In the delta negative group three had acute or unspecific changes, ten had chronic persistent hepatitis. Autopsy was not carried out in the patient who died with acute fulminant delta hepatitis.

The biopsies in delta positive addicts were carried out during the acute delta episode in five of whom three showed CAH and two acute hepatitis without signs of chronicity. One of these two was biopsied eight years later and now had CPH. In the remainder the year of delta infection was known in five, biopsies after three, three, eight, 9 and 10 years showed respectively cirrhosis, CAH, CPH, CPH and CAH. In five patients in whom the time of onset of delta infection could not be established CAH and CPH were found in two each and unspecific changes in one. In delta negative addicts biopsies were carried out one to two years after their first presentation.

The histological findings may be thus summarized: severe liver damage was found in eight of 14 delta positive HBsAg carriers, whereas the damage was only slight in all 13 delta negative HBsAg carriers.

Biopsies were not carried out in the patients with simultaneous HBV and delta infection. No one died. The long-term prognosis was the same as in acute hepatitis B without delta infection: a chronic carriership developed in 3.5 per cent (2/57) of delta infected patients and in 4.6 (25/535) of patients without delta infection.

Acute hepatitis B + delta infection. We found that in acute hepatitis B with simultaneous delta infection progression to a chronic HBsAg carrierstate does not occur more often than after acute hepatitis B without delta infection.

Similarly, Smedile et al (52) found no chronic carriers developing from 42 persons with acute delta and HBV infection.

A few reports apparently contradict these findings. Thus, in a study done in Naples (53) the over-all rate of progression into chronicity in addicts presenting with delta positive HBsAg positive acute hepatitis was over 50 per cent. As stated by the authors themselves many of these patients were probably instances of superinfection rather than coinfection. According to Farci et al (51) the great majority of such patients represents superinfection.

In a study from Milan (50) there was no example of chronicity developing in acute hepatitis positive for HBsAg and delta if HBeAg was also present, whereas an 18 per cent chronicity rate was seen in those patients with anti-HBe instead of HBeAg. This again is probably examples of acute delta infection occurring in patients who are chronically HBV infected already.

It therefore appears that true acute hepatitis B (i.e. positive for anti-HBc IgM) with acute delta coinfection carries the same prognosis as acute hepatitis B without delta.

A reservation should, however, be made for the unusual event of acute fulminant hepatitis. In our own material of over 1000 cases of acute and chronic HBsAg-positive hepatitis occurring over 15 years there is only one death due to acute fulminant hepatitis, that of delta superinfection in a middle-aged drug addict who was registered as an HBsAg carrier four years prior to his death. In the Dublin out-break (54) there was only one case of fulminant hepatitis and it was not associated with delta infection. Smedile et al. (55) on the other hand have shown that in 40 patients with HBsAg positive delta positive fulminant hepatitis originating from Italy, Greece, U.S.A., England and Sweden (one - our own case) anti-HBc IgM was demonstrable in 62.5 per cent.

The true frequency of fulminant hepatitis in simultaneous HBV and delta infection remains unknown. A hypothetical speculation is that the response to delta agent infection is not the same in the two epidemiological types of delta infection, possibly because of genetic differences as suggested by Shattock et al (56).

Acute delta hepatitis + chronic HBV infection. Several authors, mainly from Italy, have demonstrated an association between delta infection and liver damage. Some of the studies examine the prevalence of delta markers in various types of liver disease. Thus Rizzetto (24) in the original study found delta antigen with similar frequency in CAH and CPH but a subsequent study from Los Angeles (31) found an association almost exclusively with CAH.

Others have studied the frequency of various types of liver disease in patients positive (and sometimes also compared with patients negative) for delta markers. A very high prevalence of delta markers in serious liver disease such as CAH, cirrhosis and primary liver cancer was found in an Italian multicenter study (58) and in a Greek study (59). A study from Milan (60) with delta negative controls found a 94 per cent prevalence of CAH or cirrhosis in delta positive HBsAg carriers versus 61 per cent in delta negative carriers.

Finally, in a prospective study (29) of patients with CPH and CAH deterioration was found during follow-up in 77 per cent of delta positive CAH and in 16 per cent of delta negative. In this study two patients with biopsy-proven CPH were infected by the delta agent and developed CAH.

In most of the works from Italy intravenous addicts were absent or formed only a minor part (16 of 137 in the multicenter study [58], six of 192 in the Milan study [60]).

Our findings when comparing liver biopsies from 14 HBsAg positive, anti-delta positive drug addicts with biopsies from 13 delta negative addicts fully confirm these findings. A diagnosis of severe liver damage (CAH, cirrhosis, primary liver cancer) was made in eight of 14 delta positive addicts (54 per cent) whereas in all 13 delta negative addicts the findings were benign (10 CPH and three with acute hepatitis or unspecific changes).

In summary: whereas chronic HBsAg infection in addicts seems to be a histologically benign condition as long as the patient remains free of delta infection the outlook changes decidedly for the worse once delta infection supervenes. Serious liver damage is then found in more than half of the patients.

Comparison of two populations of HBsAg carriers

From a consecutive series of patients with acute and chronic hepatitis B seen 1970-1983 all chronic carriers of HBsAg were studied whose source of infection was either homosexual contact or intravenous drug addiction.

Sixteen homosexual men were seen of whom two presented with acute hepatitis B. Another two, although asymptomatic, had enzyme elevation indicative of recent infection. One was found during a pre-vaccination screening programme, the other during treatment for symptoms unrelated to hepatitis. The others were registered during various screening procedures. Average age at first contact was 34 years (22-64), the follow-up was for 4.2 years (range 1-9 years).

Similarly, 78 chronic HBsAg carriers addicted to intravenous drugs were registered (68 males, 10 females), 38 of whom had acute hepatitis when registered. The remainder were recognized during stays in prison, in rehabilitation centers, in hospital (for other disease than hepatitis) or during screening programmes elsewhere. Average age was 24.2 years (16-53), the follow-up was for 5.8 years (1-13 years).

Clinical findings

The clinical picture in these two groups showed several differences. Four out of 16 (25 per cent) homosexual men had acute hepatitis B at the start of their HBsAg carriership (confirmed by the presence of anti-HBc IgM).Of 78 drug addicts 38 presented with clinically significant acute, HBsAg positive hepatitis. However, when sera were retested in these patients two were found to have hepatitis A, five had acute delta infection, one had probably an acute alcohol hepatitis, and one was suspected of having hepatitis non-A,non-B since his first serum was positive for anti-HBe (subsequently confirmed, negative for anti-HBc IgM). In the remainder sera were available for anti-HBc IgM analysis in 19, the diagnosis of acute hepatitis B was verified in 12 and seven were diagnosed as hepatitis non-A,non-B. In 13 patients early sera were not available. In the five delta infections coinfection was found in two (positive for anti-HBc IgM) and superinfection in three (negative for anti-HBc IgM).

The clinical course was fairly uneventful in the homosexual men even when cirrhosis developed. Enzyme levels tended to become lower during follow-up, persistent normalization was seen in one only. No episode of acute hepatitis occurred after the initial episode. There was one death, the oldest patient

who died of primary liver cancer at the age of 69, five years after becoming a chronic HBsAg carrier.

In addicts there were several intervening episodes of acute hepatitis, 19 of these episodes were superinfection with the delta agent, four being hepatitis A and three hepatitis non-A,non-B. Normalization of enzymes occurred in 16 patients, in another 19 occasional normal values were seen followed by elevation at the next visit. One patient died of acute fulminant delta infection.

Serological findings

HBeAg was present in the first serum available from all the homosexual HBsAg carriers and from 67 per cent of the addicts. When the addicts were divided into those of recent origin (positive for anti-HBc IgM) and those of longer standing (negative for anti-HBc IgM) 15 were found to be recent and these were all positive for HBeAg. Follow-up demonstrated persistence of HBeAg in the homosexual men. After two years of follow-up all of 13 patients were still positive and after five years three of seven (43 per cent) were positive. In the addicts, however, HBeAg to anti-HBe conversion occurred much sooner. After two years of follow-up only 23 per cent (14/61) and after five years only 6.1 per cent (3/37) were still HBeAg positive. The rate of disappearance was the same in the total group as in the group with recent onset.

Spontaneous clearance of HBsAg occurred in 14 addicts (18 per cent) after one to eight years (mean five years). In three the clearance was preceeded by acute delta infection, in one by hepatitis non-A,non-B. Anti-HBs developed in 11. One homosexual man cleared HBsAg after eight years but this development was possibly influenced by treatment with interferon.

Delta infection markers were found in 63 per cent of those addicts who were seen after the introduction of the delta agent in 1973. Delta markers were never found among the homosexual males.

Histological findings

A total of 18 liver biopsies were carried out in 11 of the 16 homosexual patients. The findings were as follows (considering the last biopsy only): primary liver cancer (with cirrhosis and CAH) in one, CAH in five (with cirrhosis in two), CPH in three, chronic lobular hepatitis in one and acute unresolved hepatitis in one who was biopsied at the end of the first year of observation. In five patients biopsied twice or three times deterioration was seen in four.

In 25 of the addicts 27 biopsies were carried out, in addition there were two post mortems. The findings were: cirrhosis one (this patient was a middle-aged alcoholic), CAH seven, CPH 14 and acute hepatitis or unspecific changes five.

When these findings were related to the patient's delta status it turned out that all cases of cirrhosis and CAH were delta positive and that these diagnoses accounted for 57 per cent of all delta positive addicts biopsied. In the delta negative group all patients had a benign picture (10 CPH, three acute hepatitis or unspecific changes).

Discussion

Chronic carriers of HBsAg have different characteristics depending on age when infected, sex, geography, accompanying disease, immune status, type of exposure. So called healthy HBsAg carriers who occur with a prevalence of approximately 0.1 per cent in low endemicity areas like Scandinavia are believed to result from early childhood infection like in high endemicity countries of Asia and Africa. This concept is difficult to reconcile with the general non-infectivity of female HBsAg carriers of child-bearing age. The few infectious young females seen in our community have all been addicts, i.e. they are a recent phenomenon.

Subgroups like homosexual men and intravenous addicts with HBsAg carrier-rates of two to five per cent and anti-HBs prevalences of around 50-60 per cent may in global terms be said to constitute sectors of medium endemicity. In these groups the origin of the carriership is not in doubt, infection arose from sexual contact or from shared hypodermic needles. Also the time of infection may safely be placed in adulthood. This is the rationale for the present investigation of 94 chronic HBsAg carriers, 16 homosexual males and 78 intravenous addicts. They are examples of a carriership developing in adults. A number of other similar groups extensively studied elsewhere were not available to us. Thus the absence in our city of a large institution for the mentally retarded excludes one type of chronic HBsAg carriers. The remarkable absence since the 1960:s of hepatitis among patients and staff in the local renal department excludes another.

In intravenous drug addicts several workers have found a high prevalence of markers of hepatitis B. From two to 10 per cent have been found to be chronic carriers of HBsAg and 40-60 per cent to have anti-HBs or anti-HBc (61, 62, 63). Biopsy studies in addicts with or without overt signs of clinical disease have shown a high frequency of serious liver damage. American workers have reported

findings of 15-20 per cent chronic persistent or chronic aggressive hepatitis in drug users with and without hepatitis B (62). In another work in which 60 addicts were biopsied there were 35 instances of chronic persistent and 10 of chronic aggressive hepatitis,a majority of which was thought to result from HBV infection (61). However, the role of delta superinfection in causing the liver damage was not analysed in these studies.

Long-term follow-up studies of chronic HBsAg carriers have repeatedly been published but have rarely dealt with the two groups discussed here. We have not found any longitudinal study dealing exclusively with chronically HBV-infected drug addicts, probably because of inherent problems in patient compliance.

The earliest reports of increased occurrence of hepatitis B in homosexual men appeared soon after diagnostic assays for the detection of Australia antigen had come into general use (64, 65, 66) and aroused particular interest because they demonstrated that hepatitis B could be transmitted by sexual contact in the absence of blood transfusions, injections and other types of blood exposure (67). Similar prevalences of HBV markers have been found as in addicts (49, 68, 69, 70).

Information as to the degree of liver damage in homosexual men is scant. In a study of 100 chronic HBsAg carriers in London (71), of whom 77 were homosexual men, CAH was found in 29, cirrhosis in eight, and hepatocellular carcinoma in eight. On the other hand, in 10 asymptomatic homosexual males biopsied in Copenhagen (72) there was a preponderance of more benign lesions, CPH in six, non-specific reactive hepatitis in two, cirrhosis in one and acute hepatitis in one.

One of the clinically observed differences between homosexual men and drug addicts with chronic HBs antigenaemia has a ready explanation. The absence of repeated episodes of hepatitis in homosexual men when compared with addicts is in great measure due to the absence of delta infection. Introduction of the delta agent into the homosexual community might have very serious consequences. Indeed, one good reason for wide-spread hepatitis B vaccination of homosexual men is prevention of delta infection.

Why hepatitis non-A,non-B should be prevalent among addicts but not in homosexual men is not clear. According to results obtained during the New York vaccine trial in homosexual men (73) the attack rate for hepatitis non-A,non-B in homosexual men is 2.9 per cent anually versus 5.2 per cent for hepatitis A and 18-35 per cent for hepatitis B. The transmission of the non-A,non-B agent(s) might be less efficient by the nonparenteral (transmucosal) than by

the parenteral (percutaneous) route.

It should be added that although chronic hepatitis B takes a more uneventful course in homosexual men than in addicts, the spectrum of disease associated with homosexuality is wide but the conditions are often treated in venerology or proctology departments and therefore not seen in our clinic.

Our histological findings in homosexual men should be compared to biopsy findings in addicts without delta infection. In so doing we find an unexpectedly high number of serious conditions ranging from CAH to primary liver cancer, which are absent in delta negative addicts. The fact that in five patients with more than one biopsy deterioration was seen in four emphasizes the potentially serious nature of the liver damage.

The high prevalence of HBeAg and the low rate of conversion to anti-HBe has been noted by other workers (12, 14, 68, 74). It is unclear why continued viral replication for several years should be the rule among homosexual men but the exception in addicts. The implications of these findings for the spread of disease among addicts have been dealt with in an earlier paragraph.

At this point, finally, a number of factors should be listed that might possibly affect the outcome of chronic HBV infection in the two groups.

One is age. The higher average age in the homosexual group might be of importance since clinical hepatitis is often more severe and more protracted in older patients (75). In our material the two patients who died were both the oldest in their group. However, the difference in age is too small to account for the whole difference.

Another factor is the generally low standards of hygiene and poor social integration of the addicts. Homosexual men are mostly employed in white-collar work and conscious of hygiene and personal appearance. This, if anything would work towards poorer health among addicts.

The number and nature of diseases associated with homosexuality and addiction respectively is different. In addition to several types of hepatitis, staphylococcal infections are common among addicts. In the homosexual it has repeatedly been stressed in connection with the acquired immunodeficiency syndrome (AIDS) that repeated infections may reduce immunological competence.

The mode of transmission is different. The non-parenteral route in the homosexual men might be the reason for the low yearly incidence of clinical cases as compared with the addicts. Subclinical disease with consequent immunity might be the normal result of this type of transmission in which the number of infectious particles transmitted could well be lower than in percutaneous transmission. It is difficult to see, however, how this would affect the

prognosis adversely in those few homosexual men who do become chronic HBsAg carriers.

The findings of a distinctive agent responsible for the severe liver damage prevalent in delta infected addicts suggests that a like-wise unknown agent might be responsible for liver damage in homosexual men. AIDS is rare in Sweden and has not been reported in our community. On the other hand, nearby Copenhagen has the highest incidence of AIDS in any city in Europe (76). One or two cases of the lymphadenopathy syndrome are on record in Malmoe.

We conclude that although a number of hypothetical points may be raised the reason for the severity of chronic HBV-associated liver disease in homosexual men remains obscure.

Hepatitis B virus DNA

Acute self-limiting hepatitis B (group I)

Among 79 patients in whom acute hepatitis B was diagnosed on the basis of clinical and laboratory findings and temporary HBsAg positivity 56 (71 per cent) were positive for HBV DNA. DNA clearance was observed in all, in 20 of them occurring before the end of the first week. At least five with otherwise unremarkable course of disease were positive for more than one month. The longest period of positivity observed was 42 days after onset of disease.

Of the 56 HBV DNA positive patients 53 were initially also HBeAg positive. In the majority (56 per cent) loss of DNA preceded loss of HBeAg by an average of 22 days (3-9 days). Of the remainder, 12 lost the HBeAg 14 days (4-37) before DNA clearance, in the rest it was lost simultaneously with DNA.

The average age of the patients in the DNA positive group was 34.8 years while the 23 patients in whom DNA was not detected had an average age of 25.8 years. The severity of hepatitis in the two groups as expressed by the mean of maximum ALT values was the same in the two groups.

Of the 23 DNA negative patients 18 were positive for HBeAg, seven in one early sample only, in 11 it was lost after a mean of 32 days (13-11).

Since viral DNA is presumably present at some time in all patients infected with HBV an attempt should be made to explain why it was absent in 29 per cent of our patients. If a patient presents late, DNA clearance may already have been accomplished. Such a patient would have lower enzyme levels than patients presenting earlier. This was, indeed, seen in a few cases, but on the average enzyme levels were of the same magnitude in DNA positive and negative patients

and therefore late presentation would account for only a few instances of absence of DNA.

There was an age difference of 9 years between the two groups suggesting that DNA clearance is influenced by age. Indeed, DNA was almost invariably present in the middle-aged and elderly patients but absent in half of those younger than 20.

Two patients were discovered at such an early stage that repeated DNA measurements could be carried out before onset of acute hepatitis. In both the DNA maximum occurred before clinical signs and enzyme elevation had developed fully.

We conclude that infectivity in acute hepatitis B like in many other viral infections, such as hepatitis A, is a feature of the presymptomatic and early symptomatic phase of the disease. It is lost in most patients long before clearance of HBsAg, particularly when this is measured by high-sensitivity third-generation assays.

Acute hepatitis B evolving into chronicity (group II)

In the 17 patients in whom acute hepatitis B evolved into chronic HBs antigenaemia HBV DNA was persistently positive in four throughout the entire observation period of 20 months (6-57 months). These patients also retained the HBeAg throughout.

In the remaining 13 patients DNA clearance occurred after a mean period of 23 months (2-57). In one patient DNA clearance was noted within two and a half months after his first presentation. Nevertheless, chronic antigenaemia developed and persisted two years later. All the remainder were DNA positive for at least six months.

Twelve of these 13 patients were positive for HBeAg in their first sample. Loss of HBeAg occurred before DNA clearance in four (three and four months earlier in two, unknown in two). In four there was simultaneous loss of HBeAg and HBV DNA. HBeAg was retained after DNA clearance for five, five, and 12 months, respectively, in three patients. In the fourth the HBeAg still persisted 54 months after DNA clearance.

Comparing groups I and II we find that in all of the 79 cases of acute self-limiting hepatitis no patient was positive for HBV DNA for more than 42 days, whereas among the 17 cases of acute hepatitis evolving into chronic HBs antigenaemia all but one were positive for HBV DNA for at least six months. It is therefore clear that persistence of DNA is an accurate indicator of

impending chronicity.

The same forecast could have been made by means of HBeAg which remained positive for more than six months in 15 patients who became HBsAg carriers. One patient had early disappearance of DNA and HBeAg, in another the results were inconclusive. However, among the patients with acute hepatitis B eight were HBeAg positive for 42 to 110 days despite subsequent loss of HBsAg. Therefore of these two prognostic indices HBV DNA seems the more reliable.

In the four patients in whom HBeAg persisted after DNA clearance, HBe was finally lost in three (after five, five, and 12 months). The fourth patient observed for more than a decade remained HBeAg positive for 54 months after DNA clearance had been observed. The meaning of this finding is obscure.

Chronic hepatitis B without history of acute hepatitis (group III)

In 43 chronic HBsAg carriers without a history of clinical hepatitis DNA was demonstrated in 39 (91 per cent). It was absent in four of whom two were Vietnamese children.

DNA clearance was observed after a mean of 15 months (3-72) in 25 patients DNA persisted in 12 despite observation times of 49 months (6-108).

HBeAg was detected in the first serum of all the 25 patients who cleared DNA. It was subsequently lost simultaneously with DNA in eight. In another eight it was lost eight months (3-22) later than DNA, whereas five patients retained HBeAg during the entire observation period after DNA clearance of 12 months (1-22 months). In four patients further follow-up is pending.

In the 12 patients who retained DNA throughout the HBeAg was also retained in 11. In this group occurred the following unusual case of HBV DNA associated with anti-HBe:

Case history I (fig. 3)

This 25 year old male intravenous drug addict was discovered at a routine control to be HBsAg positive. Being negative for anti-HBc IgM, positive for anti-HBe and having normal enzymes he was taken to have been a chronic HBsAg carrier for some time already. After three months he developed acute delta superinfection with protracted enzyme elevation, six months later ALT was still increased by a factor 25 and biopsy showed chronic aggressive hepatitis. The patient was strongly positive for HBV DNA in all 14 available sera. He was positive for anti-HBe in an early and in a late serum and negative for HBeAg and anti-HBe in two sera obtained during the middle part of the observation period.

In the four patients in whom DNA was not found three were positive for HBeAg, one for anti-HBe.

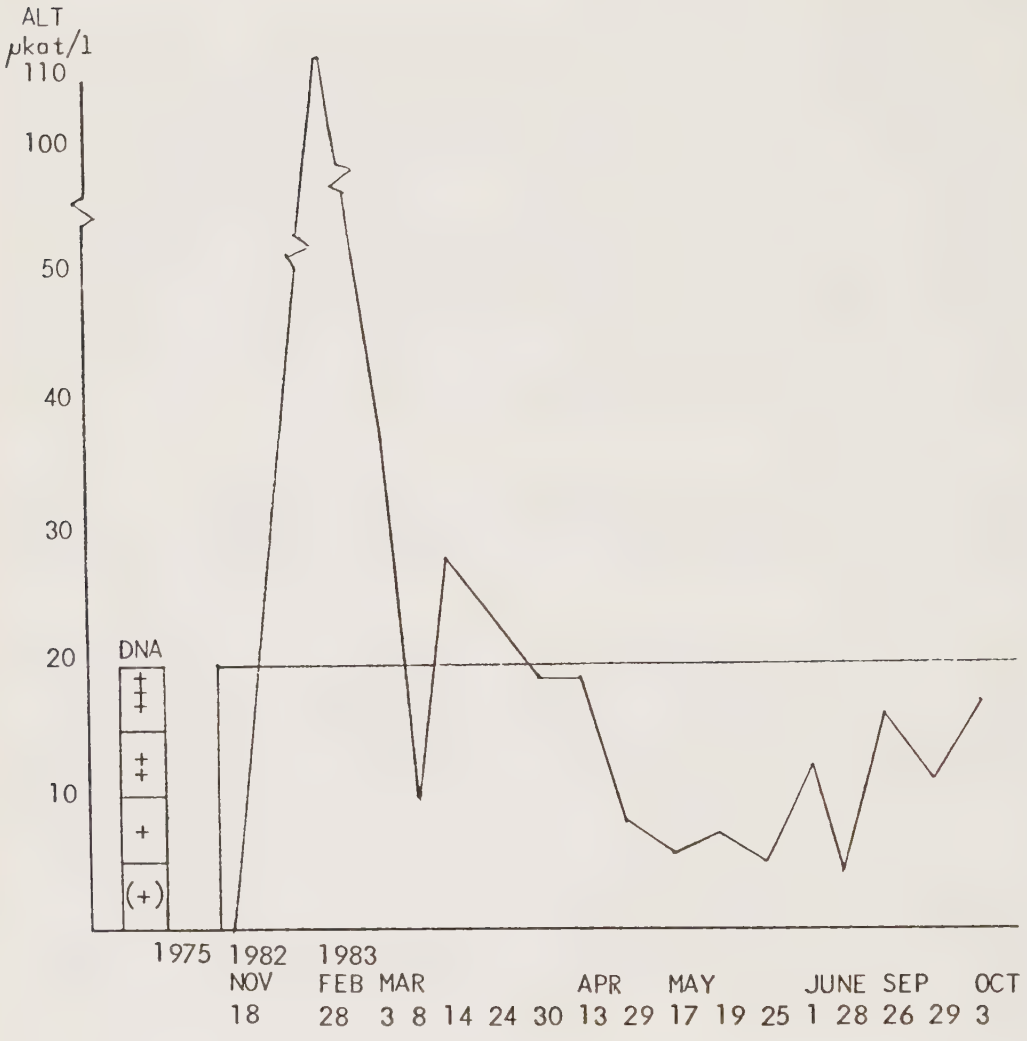

Fig. 3. Acute delta hepatitis in chronic HBsAg carrier positive for anti-HBe. Level of HBV DNA is unaffected throughout.

Previous workers in this field have mainly dealt with chronic HBsAg carriers. In the earliest report Brechot et al (17) compared one group with free (as well as integrated) HBV DNA in the liver with another with only integrated DNA. HBV DNA and HBeAg were found in the serum of the first group only and two types of the carrierstate were defined accordingly.

Bonino et al (18) found that intrahepatic HBcAg correlated 100 per cent with serum-HBV DNA, but only 86 per cent with the presence of HBeAg.

In subsequent studies (19, 20) comparable results were obtained. In later works (21, 22, 23) the discussion has focused on chronic HBsAg carriers positive for anti-HBe and with chronic liver disease. Such patients are probably infectious (21) and in the Mediterranean area mostly indicative of chronic delta infection (23).

In the present study there was a good correspondence between HBV DNA and HBeAg in the chronic HBsAg carriers in whom DNA persisted throughout the entire observation period. If groups II and III are taken together there are 16 such patients of whom 15 retained HBeAg throughout

In the 38 patients in whom clearance of DNA occurred during the observation period (13 from group II, 25 from group III) the correlation is less clear-cut. In 16 HBeAg disappeared a few months before or simultaneously with DNA but in 21 the HBeAg persisted after DNA clearance for an average of 16 months (2-54). In 11 HBeAg was subsequently lost.

The constellation HBeAg negative/HBV DNA positive was seen only in four patients for three and four months in those two in whom information was available. In two HBeAg to anti-HBe conversion was accomplished between two visits, but in two more anti-HBe was detected only after a period of negativity to both HBeAg and anti-HBe.

On the other hand, the constellation HBeAg positive/HBV DNA negative is more common. The interpretation of this finding is obscure but the clinical implication is obvious. If infectivity is defined according to the patient's HBeAg/anti-HBe status a considerable overdiagnosis of infectivity might result.

Markers of delta-infection were found in 9 HBsAg carriers. The only example of HBV DNA in a consistently anti-HBe positive HBsAg carrier was presented above (case history I and fig. 3). There was no effect on the level of HBV DNA during the acute delta episode.

Case history II (fig. 4) concerns the effect of delta superinfection on DNA level in a consistently HBeAg positive HBsAg carrier. DNA is depressed temporarily.

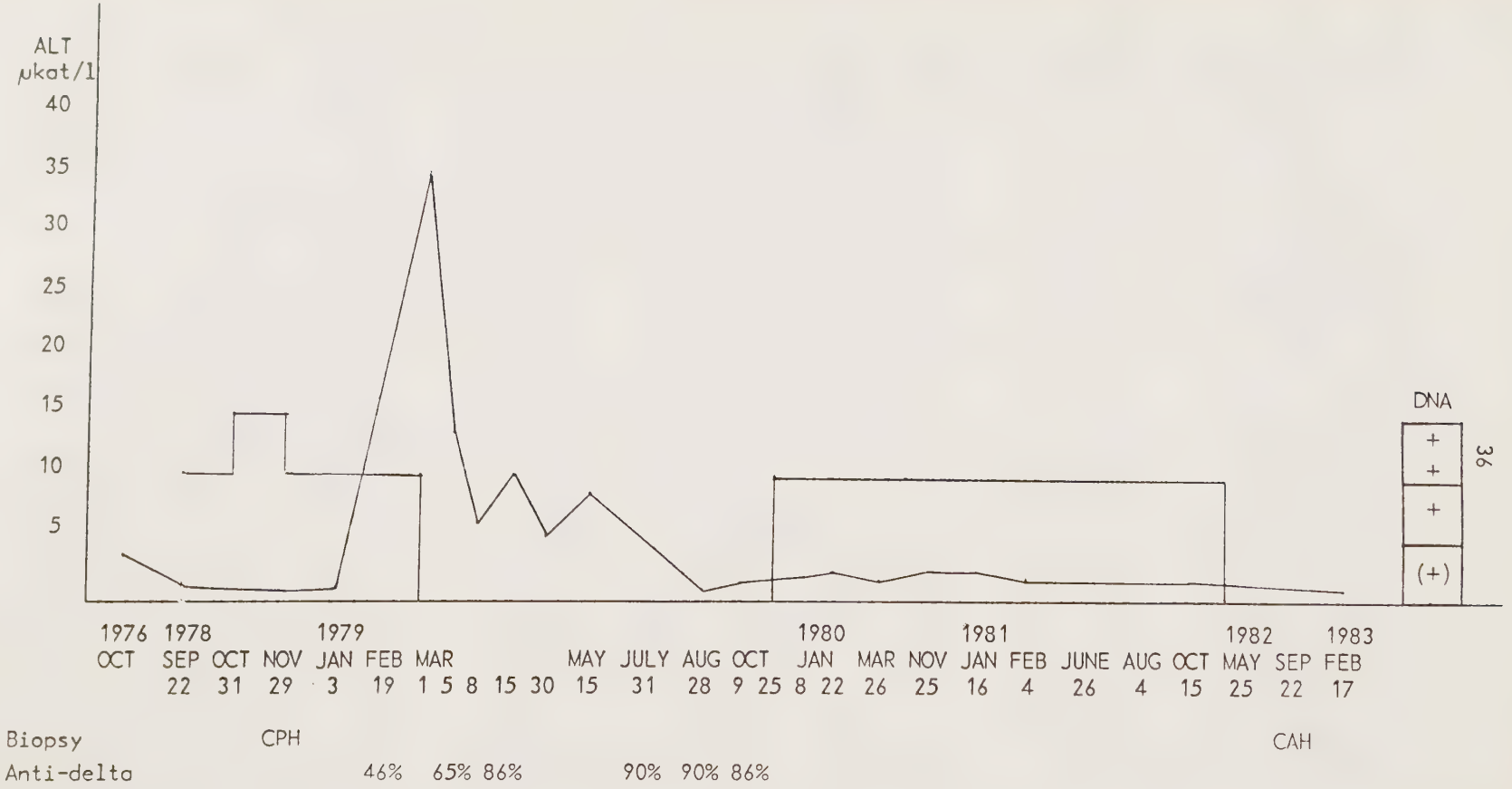

Fig. 4. Acute delta hepatitis in chronic HBsAg carrier positive for HBeAg. HBV DNA disappears during delta episode and returns after seven months.

Case history II (fig. 4)

Fig. 4 shows delta infection in an HBeAg positive HBsAg carrier (female drug addict) occurring three years after HBsAg was first detected. During the acute delta episode, January - March 1979, there was a depression of HBsAg titers and HBV DNA disappears. It returned, however, after eight months and was finally cleared two years later.

Whether these two cases represent typical patterns of the effect of delta superinfection remains to be confirmed.

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Acute Hepatitis A, B and Non-A, Non-B in a Swedish Community Studied over a Ten-Year Period

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ABSTRACT. 985 episodes of hepatitis representing 98 % of all acute hepatitis episodes found in a Swedish city during a 10-year period were analyzed for anti-hepatitis A IgM antibodies and hepatitis B surface antigen. Hepatitis A was diagnosed in 311 episodes (32 %), hepatitis B in 494 (50 %), simultaneous acute hepatitis A and B in 12 (1.2 %), and 168 episodes (17 %) were classified as hepatitis non-A, non-B. The majority of the hepatitis A cases were drug addicts (58 %), and all were concentrated in 3 outbreaks of 1–2 years duration. 16 % of all hepatitis A cases were probably imported. Hepatitis B cases decreased significantly ($p<0.001$) between the first and second half of the study period. 47 % were drug addicts. Hepatitis non-A, non-B was also dominated by drug addicts (61 %). Approximately 20 % of the cases in all 3 types of hepatitis had no identifiable source.

INTRODUCTION

During the past decade the epidemiology of hepatitis B has been well documented (10, 19, 21, 22, 23, 28). The prevalence of earlier hepatitis A infection has been studied in different populations (5, 6, 8, 15, 24, 25, 27) while the incidence of clinical hepatitis A has been less well covered (12, 14, 19, 28). Data are also accumulating on the incidence of hepatitis non-A, non-B in different communities (3, 12, 14, 16, 19, 28). In the studies mentioned above the periods of observation have been 1–3 years. In Malmö, all patient sera tested for hepatitis markers from March 1970 have been stored frozen. It has therefore been possible to perform a 10-year study of the incidence of acute hepatitis types A, B and non-A, non-B in Malmö and to correlate serological findings with epidemiological data.

MATERIAL AND METHODS

Case finding. All known cases of acute hepatitis occurring in the city of Malmö (250 000 inhabitants) from March 1970 through December 1979 were studied. Hepatitis or suspected hepatitis is a reportable disease in Sweden. The majority of cases were located primarily by utilizing this system. Unreported cases (<10 %) were located by screening hospital records. This was possible because there is only 1 hospital in the Malmö area and cases with suspected hepatitis are referred to and treated at its Departments of Infectious or Pediatric Diseases.

Criteria for acute hepatitis. Complete histories of all patients were reevaluated and only episodes with a clinical picture and biochemical findings consistent with acute hepatitis were included. The biochemical criteria were either a transient 10-fold elevation of aminotransferases or a transient 5-fold elevation of aminotransferases in the presence of hyperbilirubinemia. Cases with obstructive jaundice caused by stone, tumor and metastases as well as autoimmune liver disease were excluded. Also, the following primarily reported groups were excluded: (*a*) medicamental hepatitis (41 cases), all with a history of ingesting prescribed potentially hepatotoxic drugs; (*b*) alcoholic hepatitis (16 cases) verified by history and a massive elevation of glutamyltransferase in relation to aminotransferases; and (*c*) 3 patients with cytomegalovirus (CMV) infection and 1 with mononucleosis. Testing for these infections was limited to patients with prolonged fever and peripheral blood smears showing atypical lymphocytes.

Applying these criteria, 1 005 episodes were considered to have been acute hepatitis. In 985 (98 %) acute phase sera had been kept frozen and were available for complementary hepatitis A and B tests. These 985 episodes were seen in 915 patients; 60 of them had ≥2 episodes of hepatitis.

In most cases patients had been thoroughly questioned about probable sources of infection such as drug abuse, contact with jaundiced persons, travel abroad, transfusion and occupation. A thorough sexual history was pursued in many, but not all, patients.

Hepatitis A diagnosis. IgM antibodies to hepatitis A virus (anti-HAV IgM) were determined by an enzyme-linked immunosorbent assay (ELISA) developed by Møller and Mathiesen (17). Microtiter plates coated with anti-human IgM were used to specifically bind IgM from

Table I. *985 episodes of acute hepatitis seen in 915 patients in Malmö, Sweden, from March 1970 through December 1979*

	No. of episodes	%
Hepatitis A	311	32
Simultaneous hepatitis A and B	12	1.2
Hepatitis B	494	50
Hepatitis non-A, non-B	168	17
Total	985	100.2

patient sera. Anti-HAV IgM antibodies were then determined by consecutive incubation with a fecal extract containing hepatitis A virus (HAV), enzyme labelled anti-HAV and enzyme substrate. The enzyme reaction was measured in an automatic spectrophotometer (ELISA-reader, Flow Laboratories). Quotients of the absorbance of the individual test samples and the mean absorbance of 5 negative controls (diluent) of ≥2.1 were considered positive for anti-HAV IgM. By this technique anti-HAV IgM can still be detected between 3–6 months after onset of acute hepatitis A.

Hepatitis B diagnosis. From March 1970 through August 1970 gel diffusion (GD), and from September 1970 through January 1979 immunoelectroosmophoresis (IEOP), was used to screen for hepatitis B surface antigen (HBsAg). A commercial ELISA technique for HBsAg detection (Hepanostica, Organon Teknika) was used during the last part of the study. All cases of acute hepatitis previously found negative for HBsAg by GD or IEOP were retested by ELISA. In the majority of episodes more than one serum was tested.

Hepatitis non-A, non-B. Patients with clinical hepatitis were classified as hepatitis non-A, non-B if neither anti-HAV IgM nor HBsAg could be detected in acute phase sera.

RESULTS

Of the sera from 985 episodes with acute hepatitis which could be analyzed, 311 (32%) had anti-HAV IgM antibodies and 494 (50%) were positive for HBsAg. In 12 episodes (1.2%) the findings indicated simultaneous acute infection with both hepatitis A and B viruses. The remaining 168 episodes (17%) were classified as hepatitis non-A, non-B (Table I). Four cases of acute hepatitis A found in chronic hepatitis B carriers were counted as hepatitis A only. 60 patients had >1 registered episode of acute hepatitis (Table II). In 4 cases of acute hepatitis B, anti-HAV IgM was still present after a recent, documented episode of hepatitis A.

Age distributions in all 3 types of hepatitis were influenced to a great extent by the age profile of the drug addicts. The median age at diagnosis was 20–25 yr. Males were predominant in hepatitis A (M : F=223 : 100), to a lesser degree in hepatitis B (M : F=316 : 190). In hepatitis non-A, non-B there was an equal distribution between the sexes (M : F =87 : 81). The incidence of A, B and non-A, non-B hepatitis/3 month period is presented in Fig. 1 and the probable routes of transmission in Table III.

Hepatitis A

Three waves of hepatitis A were registered, each lasting between 1 and 2 yr. The last outbreak reached its maximum in the last quarter of 1979 and terminated during the first months of 1980 (Fig. 1, dotted area). During these 3 waves approximately 70% of all cases were intravenous drug addicts and in the periods in between practically no addicts with hepatitis A were seen. Altogether 188 (58%) of the 323 cases with hepatitis A were drug addicts. During the first half of the 1979 outbreak, opiate addicts born before 1957 dominated. Later, there was a shift to younger patients addicted to intravenous amphetamine.

In 138 (73%) of the addicts with hepatitis A, information was available concerning duration of intravenous abuse. The median duration was 2 yr (2 months–12 yr). At least 27 cases (39%) in the second and 30 (54%) in the third outbreak occurred

Table II. *No. of patients with ≥1 episodes of acute hepatitis occurring in Malmö, Sweden, from March 1970–December 1979*

Type of hepatitis	No. of patients
I. *1 episode*	
Hepatitis A	265
B	460
Non-A, non-B	118
A and B simultaneously	12
Total	855
II. *≥2 episodes*	
Hepatitis A and B	18
A and non-A, non-B	21
B and non-A, non-B	9
A, B and non-A, non-B	4
2 episodes of non-A, non-B	4
A and 2 episodes of non-A, non-B	1
B and 2 episodes of non-A, non-B	1
A, B and 2 episodes of non-A, non-B	2
Total	60

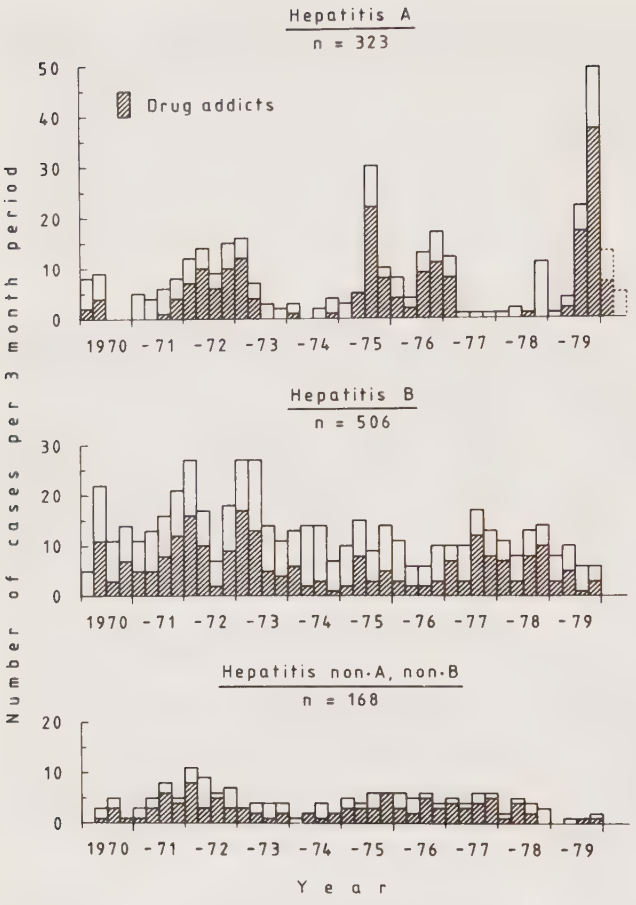

Fig. 1. No. of cases with acute hepatitis A, B and non-A, non-B per 3-month period occurring from March 1970 through December 1979 in Malmö. 12 patients simultaneously infected with both hepatitis A and B viruses are included both as hepatitis A and B. Hepatitis A cases during the first 6 months of 1980 are marked as dotted areas.

among people who had been intravenous addicts already during a previous outbreak.

51 (16%) of the hepatitis A episodes occurred after a recent journey abroad. There were no typical annual or seasonal patterns. Most of these patients had travelled to the Mediterranean region which is a major area for Swedish tourism abroad. None of them had received adequate gammaglobulin prophylaxis.

The incidence of family contact cases remained stable throughout the period. One small institutional outbreak (kindergarten) occurred in 1978. No other common source outbreak was recognized.

No cases of transfusion hepatitis A were seen. Two patients had been tattooed in the preceding 2 months but had possibly also had contacts with drug addicts. Three persons of the hospital staff had hepatitis A. None of these had worked at the Department of Infectious Diseases. Three cases occurred among homosexual men.

The cases with unknown origin of infection were equally distributed throughout the observation period with small increases during the times when outbreaks occurred among drug addicts. The only fatal case of hepatitis A was a woman aged 64 who partially recovered from hepatic coma but succumbed to gastrointestinal hemorrhage.

Hepatitis B

Of the 506 cases of hepatitis B, 299 occurred during the first 5-yr period and 207 during the second ($p<0.001$ determined by chi-square). Totally, 236 (47%) of all cases were detected among drug addicts and cases in this group occurred continuously. In 40 cases (7.9%) family members were the most probable source of infection. Hepatitis B among

Table III. *985 episodes of acute hepatitis arranged in groups reflecting probable mode of transmission*
12 patients simultaneously infected with both hepatitis A and B virus are included both as hepatitis A and B

	Hepatitis A		Hepatitis B		Hepatitis non-A, non-B	
	No.	%	No.	%	No.	%
Intravenous drug addiction	188	58	236	47	103	61
Hepatitis contact	21	6.5	40	7.9	7	4.2
Homosexuality	3	0.9	28	5.5	1	0.6
Blood transfusion	0	0	13	2.6	6	3.6
Factor VIII concentrate	0	0	1	0.2	4	2.4
Tourism abroad	51	16	19	3.8	13	7.7
Tattooing	2	0.6	6	1.2	0	0
Hospital work	3	0.9	32	6.3	1	0.6
Unknown	55	17	131	26	33	20
Total	323		506		168	

homosexual men (28 cases, 5.5%) occurred in small clusters. 19 cases (3.8%) were found among people who had been abroad during the last 6 months. There were 13 cases (2.6%) of post-transfusion hepatitis type B. 10 had received blood in Malmö and 3 elsewhere. One patient had been treated with factor VIII concentrate. Hospital staff was more often affected in the first 5 yr during which 22 of the 32 cases occurred. There were no hepatitis B infections in either staff or patients at the Dialysis Department during the observation period. In about 1/4 of all cases with acute hepatitis B the route of transmission remained unknown. One fatal case occurred; a 21-year-old female drug addict whose liver failure may have been provoked by a heavy intake of alcohol during convalescence.

Hepatitis non-A, non-B

168 cases of acute hepatitis were classified as hepatitis non-A, non-B (17%). Of these, 103 (61%) were drug addicts and cases in this group occurred almost continuously. Family contact cases, cases among homosexual men and among hospital staff were few. 13 occurred among people who recently had been abroad. Six patients had contracted non-A, non-B hepatitis after blood transfusions and 4 after receiving factor VIII concentrate. In 33 patients the route of transmission was unknown. No fatal cases were seen.

DISCUSSION

The patients in this study should include the vast majority of acute hepatitis cases that have sought medical care in the city of Malmö during a 10-yr period. The biochemical criteria used in this study for the diagnosis of acute hepatitis were established to include cases which would be readily recognized by most physicians at the time of disease.

The average attack rate of acute hepatitis in Malmö was 0.04%/person/yr. This figure is somewhat higher than the reported Swedish average of 0.02–0.03% during the same period (18). The reason for the higher incidence in this urban area may be attributable to the larger number of drug addicts in a city population. In 1979 the number of intravenous drug addicts in Malmö was estimated to be 750–1 000 (20). The number was probably rising until 1975 but has thereafter remained stable (W. Rune, personal communication). In 1970 almost all intravenous addicts used amphetamine but more recently the number of opiate abusers has increased. In 1979 about 1/3 of the addicts used opiates, 2/3 used amphetamine and <10% combined these drugs (20). Homosexuality among drug addicts is so far practically unknown in Malmö. Among drug addicts the attack rate of diagnosed acute hepatitis was 5%/person/yr.

Only 2 (0.2%) of the 1 005 cases in the study had a fatal outcome and it is unlikely that other fatal cases have been treated without being reported or diagnosed. No person with signs of fulminant hepatitis died outside hospital during the study period (G. Voigt, personal communication).

The distribution of different types of acute hepatitis is similar to results obtained in Gothenburg covering the years 1974–1976 (19). A Copenhagen study from September 1976–January 1978 (14) showed a higher incidence of hepatitis A. During the first part of that period there was a concen-

tration of hepatitis A cases in Malmö. The occurrence of hepatitis A among drug addicts differs greatly in populations studied so far. Incidence studies from Hannover (16), Copenhagen (12, 14) and Zürich (28) as well as prevalence studies from Melbourne (9) have not indicated that drug addicts run an increased risk of contracting hepatitis A. The present 10-yr study from Malmö as well as a 3-yr study from Gothenburg (19) show a high incidence of hepatitis A among drug addicts. As our study indicates the number of drug addicts with hepatitis A can vary greatly from one period to another. If, therefore, the study period is too short, drug addicts with hepatitis A may not be found.

The high number of drug addicts with hepatitis A raises the question whether hepatitis A can be transmitted parenterally. It is known that hepatitis sera from the incubation period can infect both humans (11) and marmosets (13). The duration of viremia has not been well defined although Krugman et al. (11) have shown that sera drawn as early as 2–3 weeks prior to the onset of clinical disease were infectious. If hepatitis A is introduced into a group of drug addicts who share injection needles it seems reasonable to assume that the spread of hepatitis A virus, at least to some extent, can occur parenterally. Other factors which may explain why intravenous drug addicts in each outbreak of hepatitis A were the dominating group are poor personal hygiene and poor housing conditions, especially among opiate addicts, and the more pronounced tendency towards group activities among amphetamine abusers. Cyclic occurrence is well known in infectious disease but has not been previously described for hepatitis A among drug addicts. If drug addicts live in closed subcommunities as they seem to do to some extent, it is possible that during an outbreak of hepatitis A, herd immunity gradually develops in infected groups. Unless the infection is transmitted to other susceptible groups, it dies out, since there is neither chronic carriership of hepatitis A virus nor an endemic supply of virus. New people continuously start to use drugs. As most of them are young, they are likely to lack immunity to hepatitis A (8, 27). These persons together with older addicts, not infected in previous outbreaks, provide a gradually increasing pool of susceptibles that can be infected if the virus is reintroduced.

The high incidence of hepatitis A and the long-lasting outbreaks among drug addicts may also be linked to difficulty in identifying exposed contacts in the population of addicts. Thus, gammaglobulin cannot be given as effectively to this group as to others.

In the present study none of the 19 post-transfusion cases were due to hepatitis A which agrees with epidemiologic studies on transfused and dialysed patients (1, 7, 26). This may reflect the short duration of viremia in hepatitis A when compared with the long duration in chronic hepatitis B and non-A, non-B carriers. The prevalence of anti-HAV among hemophilia patients in Malmö who have received factor VIII and IX concentrates is identical to the prevalence in the normal Swedish population (B. G. Hansson, unpublished results), indicating that HAV is not a contaminant of clotting factor concentrates, which has been shown with B and non-A, non-B hepatitis viruses.

Corey and Holmes (4) have described a high incidence of hepatitis A among homosexual men. The 3 cases found in this study may represent an underestimate since hepatitis patients during the early stages of the study were not asked about homosexuality.

Hepatitis A was not a nosocomial problem during the observation period at the Department of Infectious Diseases, despite the fact that hepatitis cases were nursed in common wards with limited access to separate toilets. This finding is in accordance with a study by Belfrage and Cederberg (2) covering 1 113 hospitalized cases of hepatitis in Malmö between 1948 and 1969. During that time, when separate toilets were rarely available, only 1 case of hepatitis A was found that had possibly been transmitted from a fellow patient.

Hepatitis B occurred continuously and without the distinct periodicity of hepatitis A. The reason is probably that chronic hepatitis B virus carriers provide a reservoir of virus, especially among drug addicts, homosexual men and certain immigrant groups. Drug addicts represented almost half of the hepatitis B cases which is comparable to other Scandinavian populations studied (14, 19). Sexual transmission was probable in many of the family contact cases and, in some of the imported cases, and was obvious among homosexuals. The decline of hepatitis B cases among hospital staff can be explained by better awareness of the hepatitis risks when handling emergency cases and blood samples.

As there is no serologic method for identifying

non-A, non-B hepatitis the diagnosis is one of exclusion. It is sometimes difficult or impossible to distinguish between liver damage of viral origin and such secondary to other insults. This is especially true for people who use drugs and/or alcohol. Hepatitis non-A, non-B diagnosis in the present study did, in some but not all cases, exclude infections by CMV and Epstein Barr virus. The reason for not testing all patients for these infections were the findings by other investigators that these viruses play only minor roles in clinical hepatitis (3, 19).

The pattern of the non-A, non-B hepatitis incidence resembled that of hepatitis B but the total number was 3 times less than the hepatitis B cases. The non-A, non-B cases in this study represented the same fraction of the total number of hepatitis patients as reported from other populations (3, 14, 16, 19, 28). Drug addicts and cases of unknown origin predominated. A limited number of post-transfusion cases were found, and so far only 1 episode of hepatitis non-A, non-B in hospital staff was recognized.

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Infection with Delta Agent in Sweden: Introduction of a New Hepatitis Agent

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To investigate the epidemiology of infection with delta (δ) agent in a Swedish city, 181 chronic carriers of hepatitis B surface antigen (HBsAg) and 599 patients with acute, self-limited hepatitis B were analyzed for δ antigen and antibody to δ antigen (anti-δ). The study covered the period from 1970 to 1981. The δ agent was found to have been introduced to this population in 1973. Markers of infection with δ agent were almost exclusively found in intravenous drug addicts and their close contacts. The proportion of drug addicts who were chronic HBsAg carriers with anti-δ increased with time and reached 72% in 1979–1981. An episode of acute hepatitis was frequently seen in connection with seroconversion to anti-δ. Among the domestic cases of acute, self-limited hepatitis, no simultaneous infections with hepatitis B virus and δ agent were found before 1975. From 1975 to 1980, between 18% and 44% of the drug addicts with acute hepatitis B were also infected with δ agent.

The delta (δ) antigen was first detected by immunofluorescence in liver biopsy specimens from carriers of hepatitis B surface antigen (HBsAg) in Italy [1]. Experimental studies in chimpanzees have shown that the antigen is associated with a transmissible hepatitis agent (δ agent), which for its replication requires helper functions from hepatitis B virus (HBV). When transmitted to chimpanzees that are chronic HBsAg carriers, the δ agent caused acute hepatitis [2]. In human carriers of HBsAg infection with δ agent also causes an episode of acute hepatitis which frequently progresses to chronic hepatitis [1, 3, 4]. During the acute phase of infection with δ agent, δ antigen has been found in the serum capsulated by HBsAg in 35–37-nm particles. These particles also contain the putative genome of the δ agent, a low-molecular-weight RNA [5].

The epidemiology of infection with δ agent has been studied through the prevalence of antibody to δ antigen (anti-δ). Anti-δ has been found among all populations in the world studied so far, but it is exclusive to persons with markers of HBV infection. The prevalence is high in chronic HBsAg carriers in Italy, especially in the southern area. In other parts of the world, the prevalence of anti-δ is very low except among polytransfused hemophiliacs and drug addicts [3, 6, 7]. In the present study we analyzed all known chronic HBsAg carriers during 12 years and most patients with acute, self-limited hepatitis B in a Swedish city during an 11-year period for markers of infection with δ agent.

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Materials and Methods

Chronic HBsAg carriers. Of 181 chronic HBsAg carriers registered between 1970 and 1981 who were analyzed for markers of infection with δ agent, 80 were drug addicts, seven were hemophiliacs, 12 were homosexual men, and 82 had unknown sources of infection. All patients had received medical care at the Department of Infectious Diseases at Malmö General Hospital or at the hospital unit of the city jail in Malmö, Sweden. These units take care of practically all of the persons with acute hepatitis in the city of Malmö (population, 250,000). Of those patients, 128 were Scandinavians and 53 were immigrants from 22 other countries around the world. From each of these HBsAg carriers, an average of nine blood samples had been collected and stored frozen.

Acute hepatitis B. Of all of the cases of acute, self-limited hepatitis B occurring in Malmö from 1970 to 1980, we analyzed 599 patients (94% of the registered cases) during this period for δ antigen and anti-δ. Of these patients, 291 were iv drug addicts and 308 had no recognized drug abuse.

Solid-phase radioimmunoassay (RIA) for δ antigen in serum. The solid-phase RIA for δ antigen in serum was performed as described [8] with minor modifications. Polyvinyl microtiter plates (Cooke Engineering, Alexandria, Va.) were coated overnight with a 1 : 1,000 dilution of a standard antiserum to δ antigen (Dr. Mario Rizzetto, Turin, Italy). The specificity of this antiserum has been carefully analyzed [3]. After four washes with distilled water, 25 μl of serum and 25 μl of 0.6% Nonidet® P40 (BDH Chemicals, Poole, England) were added to each well, and the plate was incubated overnight at room temperature (about 22 C). After washing with distilled water, δ antigen bound to the plate was detected after incubation for 3 hr at 37 C with ^{125}I-labeled anti-δ. Unbound tracer was removed by washing four times with distilled water. The wells of the plate were cut apart with scissors and transferred to γ counting tubes. In each test one positive and five negative controls were included. The quotients of the cpm of individual test samples and the mean cpm of the negative controls (P/N values) were calculated. Values of ⩾2.1 were considered positive for δ antigen.

Blocking RIA for anti-δ. The blocking RIA for anti-δ was performed as described by Rizzetto et al. [3] with minor changes. Microtiter plates coated with the standard antiserum to δ antigen were incubated overnight at room temperature with δ antigen prepared from human liver obtained at autopsy (Dr. Mario Rizzetto). A large proportion of the hepatocyte nuclei had been found to be positive for δ antigen by immunofluorescence but negative for hepatitis B core antigen by immunofluorescence and electron microscopy [8]. The δ antigen was prepared as described [8]. It was extracted by homogenizing 1 g of liver in 6 M guanidine-HCl and stirring on a magnetic stirrer for 3 hr at room temperature, and the remaining cell debris was eliminated by centrifugation at 1,500 *g* for 5 min. The δ antigen–containing supernatant was used in a final dilution of 1 : 50. After the plate was washed four times with distilled water, 50 μl of serum diluted 1 : 10 in phosphate-buffered saline was added to each well. The plate was incubated for 3 hr at 37 C, washed, and incubated for another 3 hr at 37 C with ^{125}I-labeled anti-δ. After washing, the individual wells were analyzed for ^{125}I in a γ counter. One positive and five negative controls were included in each test. Sera that blocked ⩾50% of the binding of radiolabeled anti-δ obtained with the negative controls were considered positive for anti-δ.

RIA for IgM anti-δ. The RIA for IgM anti-δ was performed according to the principle first described by Flehmig [9]. Microtiter plates were coated overnight at room temperature with rabbit antibody to human IgM (Dako, Copenhagen) diluted 1 : 10,000 in 0.08 M carbonate-bicarbonate buffer (pH 9.6) and postcoated with 1% bovine serum albumin in the same buffer. After washing, 50 μl of the serum specimens diluted 1 : 100 in 1% cord serum that was negative for anti-δ was added and incubated for 4 hr at 37 C. After another washing procedure, 50 μl of the same standard δ antigen preparation as was used in the blocking RIA for anti-δ was added. The plate was incubated at room temperature overnight and then washed. The amount of bound δ antigen was measured by incubating with ^{125}I-labeled anti-δ for 3 hr at 37 C, washing, and measuring the radioactivity in the individual wells in a γ counter. One positive and five negative controls were included in each test. P/N values of ⩾2.1 were considered positive for IgM anti-δ.

Tests for HBV markers. Levels of HBsAg, antibody to HBsAg, hepatitis B **e** antigen, and antibody to hepatitis B **e** antigen were measured with RIA kits from Abbott Laboratories (North Chicago, Ill.). The solid-phase RIA used for determination of levels of IgM antibody to hepatitis B core antigen (IgM anti-HBc) has been described by Widell et al. [10].

Results

Chronic HBsAg carriers. The last serum sample drawn from each of the 181 chronic HBsAg carriers was tested for anti-δ. Forty-one of the 80 drug addicts and three of the 101 nonaddicts were positive for anti-δ. Of the anti-δ–positive nonaddicts, one was a hemophiliac who was found to be positive for HBsAg and anti-δ in 1975 at the age of one year without having had clinical hepatitis; the

Table 1. Prevalence of antibody to delta agent (anti-δ) in drug addicts who were chronic carriers of hepatitis B surface antigen in Malmö, Sweden, analyzed during three-year periods.

Period	No. with anti-δ/ no. analyzed (%)
1970–1972	0/26
1973–1975	12/37 (32)
1976–1978	27/54 (50)
1979–1981	41/57 (72)

NOTE. There was a total of 80 patients available for study between 1970 and 1981.

other two were immigrants from Turkey and Germany. Neither of the latter had a known history of hepatitis. They were both positive for anti-δ from the first serum samples available (drawn in 1973 and 1979, respectively).

An analysis of the serial samples collected from all of the 80 drug addicts who were HBsAg carriers showed the first seroconversion to anti-δ in 1973. The analysis of the anti-δ-positive and -negative addicts was divided into three-year periods. In each of these intervals, sera were available from a varying number of the 80 patients. The proportion of carriers positive for anti-δ increased with time, reaching 72% in 1979–1981 (table 1). In 10 cases, the first sera available were already positive for anti-δ, whereas seroconversion from negative to positive for anti-δ was observed in 31 patients. An episode of acute hepatitis was seen in connection with the seroconversion to anti-δ in 21 of these patients. In six patients, δ antigen could be detected in one or two acute-phase sera (with P/N values of between 3.1 and 47).

Examples of serologic patterns. Figure 1 shows the course of infection with δ agent in one of the chronic HBsAg carriers. The onset of the patient's HBV infection with acute hepatitis, the presence of hepatitis B **e** antigen, and a high titer of IgM anti-HBc (10^{-5}) occurred in 1970. In 1973 the patient had another bout of hepatitis. During the first week after the onset of jaundice, the amount of HBsAg in serum dropped significantly. At the same time anti-δ of the IgM class was detected in an increasing titer. The blocking RIA for anti-δ gave positive results somewhat later. However, δ antigen could not be detected in any serum specimen. About one month after the onset of illness, the HBsAg titer started to return to its

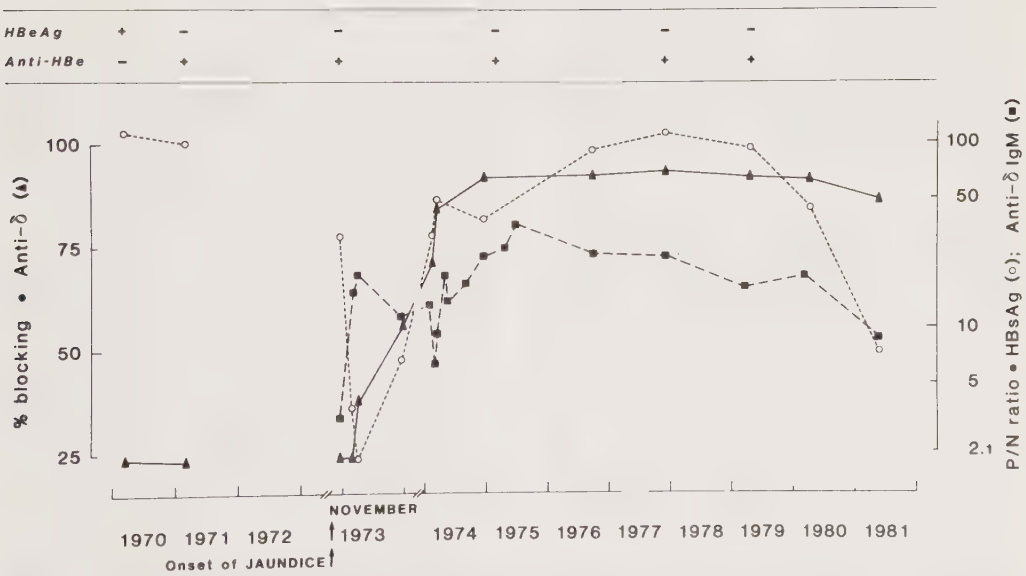

Figure 1. Infection with delta (δ) agent in a chronic carrier of hepatitis B surface antigen (HBsAg). The following serum dilutions were used in the different tests: antibody to δ antigen (anti-δ), 10^{-1}; IgM anti-δ, 10^{-2}; and HBsAg, 10^{-3}. HBeAg = hepatitis B **e** antigen; anti-HBe = antibody to HBeAg; and P/N = positive/negative control ratio.

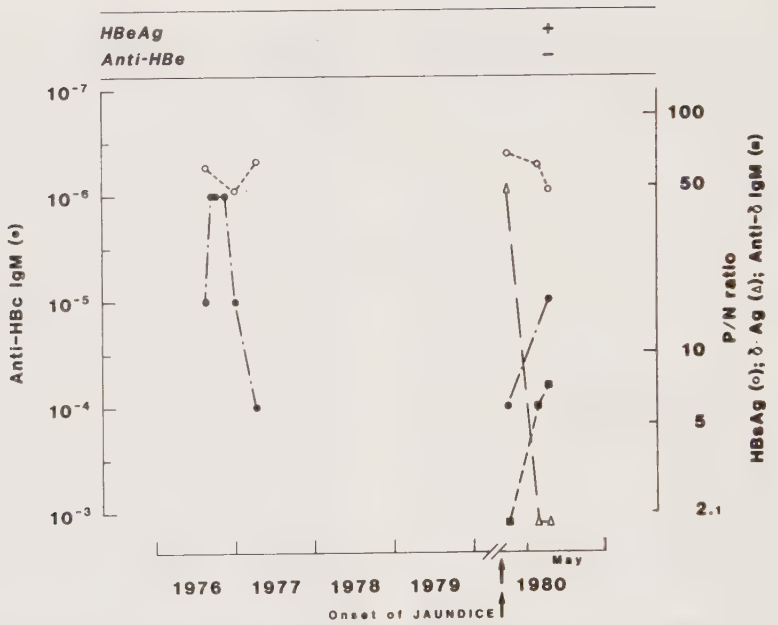

Figure 2. Fulminant hepatitis associated with infection with delta (δ) agent in a chronic carrier of hepatitis B surface antigen (HBsAg) in Sweden. The following serum dilutions were used in the different tests: δ antigen (Ag), 1 : 2; IgM antibody to δ Ag (anti-δ), 10^{-2}; and HBsAg, 10^{-3}. HBeAg = hepatitis B **e** antigen; anti-HBe = antibody to HBeAg; anti-HBc = antibody to hepatitis B core antigen; and P/N = positive/negative control ratio.

previous level. IgM anti-δ persisted during the remaining observation period of eight years.

The δ hepatitis in one of the HBsAg carriers had a fulminant course (figure 2). The patient became infected with HBV four years before becoming infected with δ agent. Hepatitis B **e** antigen persisted until the last serum sample was drawn. After the onset of the second illness, there was a tendency for the amount of HBsAg in serum to decrease. IgM anti-HBc was detected at titers of 10^{-4} and 10^{-5} four and 15 days after the onset of jaundice, respectively. Delta antigen was detected in one serum sample drawn four days after the onset of jaundice. Twelve days later, IgM anti-δ was found. After five more days, the patient died. Anti-δ was never detected by the blocking RIA.

Two of the HBsAg carriers started with simultaneous infections with HBV and δ agent. From one of them, previously drawn sera negative for HBsAg and anti-HBc were available; anti-δ was first detected six weeks after the first HBsAg-positive sample. The second patient is described in figure 3. HBsAg could be detected from the first serum sample available. In the acute phase of hepatitis, the amount of HBsAg increased initially. The titer of IgM anti-HBc started at 10^{-6}, and within nine months it had decreased to $<10^{-3}$. Delta antigen could be detected in the second and third sera drawn with a one-week interval. After a four month's gap of observation, anti-δ was detected both by the blocking RIA and by the specific IgM test. The titer of IgM anti-δ increased during the observation period of three years.

Cases of acute, self-limited hepatitis B. Table 2 lists all of the cases of simultaneous acute infections with HBV and δ agent found. The first case occurred in 1973 in a patient who four months previously had received blood transfusions in Spain. Beginning in 1975, 39% of the drug addicts with hepatitis B were also infected with δ agent. During this period four cases appeared among nonaddicts. Two of these persons had probably been infected through sexual relations with drug addicts and the other two by nonsexual contact

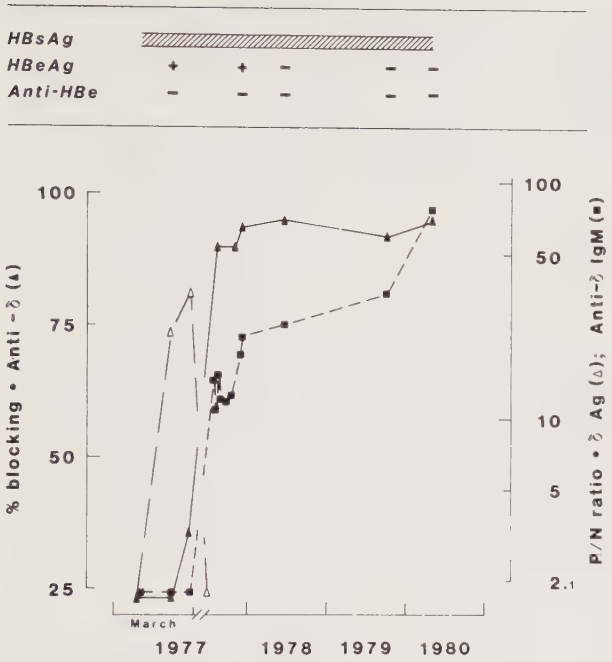

Figure 3. Simultaneous onset of infections with hepatitis B virus and delta (δ) agent leading to chronic carriership of hepatitis B surface antigen (HBsAg) in a patient. The following serum dilutions were used in the different tests: δ antigen (Ag), 1 : 2; antibody to δ Ag (anti-δ), 10^{-1}; and IgM anti-δ, 10^{-2}. HBeAg = hepatitis B e antigen; anti-HBe = antibody to HBeAg; and P/N = positive/negative control ratio.

with friends who were drug addicts. Of the total of 55 patients infected with δ agent, δ antigen followed by anti-δ was found in 22 (40%), whereas only anti-δ developing during convalescence was found in 29 (53%). In four cases (7.3%), δ antigen was the only marker of infection with δ agent because convalescent-phase sera had not been collected.

Serologic pattern. The serologic pattern in one of the patients with acute, self-limited hepatitis caused by simultaneous infection with HBV and δ agent is shown in figure 4. HBsAg and δ antigen were found during the first week and the first five weeks after the onset of illness, respectively. IgM anti-δ was first detected two weeks after the onset of illness, peaked in titer after another two weeks, and became undetectable two months later. Anti-δ was still found after one year.

Discussion

In the present study we demonstrated the introduction of a new pathogenic infectious agent, δ agent, to a Swedish population.

The first domestic patients with markers of infection with δ agent were two Swedish drug addicts who were brothers, both with acute δ-associated hepatitis in 1973. They had been known to be chronic HBsAg carriers for a few years. In the same year anti-δ was also found in a non–drug ad-

Table 2. Incidence of infection with delta agent in patients with acute, self-limited hepatitis B in Malmö, Sweden, during 1970–1980.

Year	Drug addicts	Nonaddicts
1970	0/17	0/41
1971	0/40	0/34
1972	0/40	0/29
1973	0/51	1/31 (3.2)
1974	0/15	0/27
1975	6/16 (38)	0/36
1976	5/11 (45)	2/27 (7.4)
1977	20/45 (44)	1/23 (4.3)
1978	12/30 (40)	0/24
1979	3/17 (18)	1/23 (4.3)
1980	4/9 (44)	0/13

NOTE. Data are no. of patients infected with delta agent/no. of patients analyzed (%).

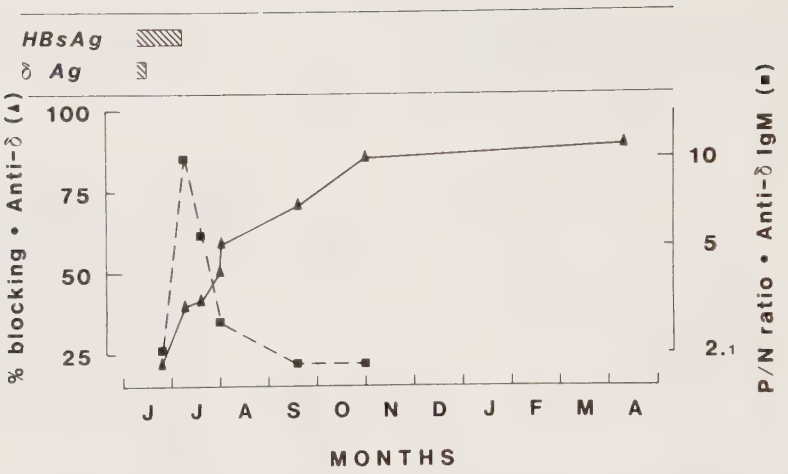

Figure 4. Acute, self-limited hepatitis associated with simultaneous infections with hepatitis B virus and delta (δ) agent in a patient. The following serum dilutions were used in the different tests: antibody to δ antigen (anti-δ), 10^{-1}; and IgM anti-δ, 10^{-2}. HBsAg = hepatitis B surface antigen; δ Ag = δ antigen; and P/N = positive/negative control ratio.

dict immigrant from Turkey who had no symptoms of acute hepatitis.

After 1973 an increasing proportion of the drug addicts who were chronic HBsAg carriers were found to be infected with δ agent, half of them with acute δ hepatitis. In total, seven of the 41 HBsAg carriers with δ agent markers came from non-Scandinavian countries. It has, however, not been possible to prove whether the δ agent was introduced by an immigrant from a country where it is prevalent or by a Swedish drug addict after visiting such an area.

Although three cases of acute δ hepatitis occurred in chronic HBsAg carriers during 1973 and 1974, no patient with acute, self-limited hepatitis caused by simultaneous infection with HBV and δ agent was seen until 1975, except for the patient with posttransfusion hepatitis in 1973 acquired in Spain. It is noteworthy that infection with δ agent has not yet spread outside the community of iv drug addicts and their close contacts. This observation confirms the previous observation that the populations of homosexual men and iv drug addicts of Malmö are not overlapping to any significant degree [11].

All of the available data show that δ agent can only replicate in the presence of HBV infection. Patients with acute, self-limited HBV and δ hepatitis can, therefore, transmit δ infection only during a limited interval. On the other hand, chronic HBsAg carriers who have been infected by δ agent seem to become chronically infected also with this agent, as shown both by the presence of δ antigen in liver [1] and by the ability of serum to transmit the infection [2]. The continuous presence of IgM anti-δ in the HBsAg carriers infected with δ agent also indicates chronic infection with δ agent as compared with the short duration of IgM anti-δ during acute, self-limited HBV and δ hepatitis. It is therefore not surprising to find that once the δ agent had been introduced into the drug addict population, the prevalence of anti-δ among the chronic HBsAg carriers increased, these individuals being a reservoir of δ agent. If introduced into another group of persons with frequent possibilities to contract and transmit viral hepatitis (like homosexual men), the same spread of δ agent in that population could be expected to take place.

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Clinical aspects of delta infection

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Abstract

The clinical features of delta infection were analysed retrospectively in 191 hepatitis B surface antigen (HBsAg) carriers and 592 cases of acute hepatitis B seen over 11 years in the Swedish town of Malmö (population 250 000). With a few exceptions delta infections occurred exclusively in drug addicts.

In the chronic HBsAg-carriers the most common clinical manifestation was an episode of acute hepatitis, which in some individuals became severe with a pronounced rise in serum alanine aminotransferase activity for many months. During the period of delta infection the HBsAg titre was lowered and in three out of 26 cases the patient lost HBsAg altogether and developed hepatitis B surface antibodies (anti-HBs). In one patient the acute hepatitis due to delta infection was fulminant and fatal. In patients with acute hepatitis B the clinical picture did not distinguish between those with and without simultaneous delta infection. The frequency with which acute hepatitis B was succeeded by a chronic carrier state was the same whether or not the patient was infected simultaneously with the delta agent.

The discovery of the delta agent has improved understanding of the natural history of chronic hepatitis B

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infection in drug addicts. Thus, instances of acute hepatitis in a chronic carrier, previously termed hepatitis non-A, non-B, may actually be episodes of delta infection.

Introduction

The delta antigen/antibody system was first described by Rizzetto and coworkers[1] in Italy in 1977 and has later been associated with a transmissible hepatitis agent, the delta agent, which can cause infection only when the hepatitis B virus genome is present. The delta agent is found all over the world.[2] It was first discovered in southern Italy, where it is endemic and associated with serious liver damage,[1] while in Northern Italy, as well as elsewhere in the world, it is prevalent among drug addicts (and haemophiliacs).[2]

Infectivity studies have shown that chimpanzees are susceptible to delta infection.[3] The inocula contained both hepatitis B virus and delta agent. In animals without previous markers of hepatitis B a self-limiting acute hepatitis developed with markers of both hepatitis B virus and delta infection, the incubation time being 3-4 weeks when highly infectious material was used and 12-13 weeks with less infectious material. In chronic HBsAg-carrying chimpanzees acute self-limiting hepatitis (with a biphasic enzyme pattern in one) was seen. The HBsAg titre was temporarily lowered before delta antibodies developed.

A previous seroepidemiological analysis[4] of about 600 cases of acute type B hepatitis and nearly 200 chronic carriers of HBsAg found in Malmö, Sweden, between 1970 and 1981 showed that delta infection was present almost exclusively in drug addicts. The infection was introduced into this town in 1973. From 1975 onwards about half of all cases of acute type B hepatitis occurring among drug addicts were also infected with the delta agent. Among intravenous drug addicts registered as chronic carriers of HBsAg by 1981, 73% were also carrying markers of delta infection.

Patients and methods

The study population comprised 592 cases of acute hepatitis B seen in Malmö (population 250 000), Sweden, during 1970-81 and 191 chronic carriers of HBsAg registered during the same period. Most patients attended the infectious diseases clinic at the General Hospital, the only one in the town. The remaining cases derived from the town prison, which is a point of referral for sick prisoners in southern Sweden. All files at the hospital and at the prison were

re-examined for details of clinical history and laboratory investigations.

Patients were diagnosed as having acute hepatitis B if they had transitory HBsAg during an acute self-limiting disease with a more than five-fold increase in serum alanine aminotransferase or clinical jaundice or both. Four subclinical cases with only transitory HBsAg were included. Forty-six patients were lost to follow-up before HBsAg titres had become negative; in these cases diagnosis was based on enzyme patterns and clinical criteria only. A chronic carrier was defined as a patient who was positive for HBsAg for more than six months. In 22 instances where follow-up was shorter than six months a carrier state was presumed when indicated by the enzyme pattern and the history of the patient. In chronic carriers of HBsAg who had acute delta hepatitis when first seen at the hospital a simultaneous diagnosis of acute hepatitis B was made only if hepatitis B core antibodies (anti-HBc) of the IgM class were present according to certain criteria.[5] Sera from all patients had been stored at −20° and were retrospectively analysed for markers of delta infection.

Tests for markers of delta infection—Solid phase radioimmunoassay for delta antigen in serum and blocking radioimmunoassay for anti-delta were performed as described.[4]

Tests for markers of hepatitis B—HBsAg, anti-HBs, HBeAg, and anti-HBe were measured by commercial radioimmunoassay kits (Abbott laboratories, North Chicago, Illinois, USA). Anti-HBc-IgM was determined by solid phase radioimmunoassay.[5]

Results

CHRONIC CARRIERS OF HBSAG

Among 191 patients delta markers were found in 45, 42 of whom were intravenous drug addicts (table I). In one fatal case delta antigen only was found; in 44 delta antibody was present and in six both antigen and antibody. By serial analysis of frozen sera the time of conversion to anti-delta was established in 26 patients. Evaluation

TABLE I—*Presence of delta markers in different groups of chronic HBsAg-carriers*

	Delta markers		Total
	Present	Absent	
Addicts	42	37	79
Homosexuals	0	15	15
Haemophiliacs	1	7	8
Others (with known source of infection)	0	28	28
Unknown source of infection	2	59	61
Total	45*	146	191

*One positive for delta-antigen, 44 for anti-delta.

of clinical symptoms at the time of conversion showed that the most common clinical manifestation of delta infection was an episode of acute hepatitis with a rise in serum enzyme activity lasting for up to two months. This was seen in 17 patients. In another eight patients the acute episode was prolonged, lasting up to one year. Finally, in one case the course was fulminant and fatal (table II).

In three patients the enzyme and bilirubin patterns were biphasic. The findings were not otherwise noteworthy. In two patients the acute hepatitis was of the prolonged type; delta antigen was present at the time of the first peak and delta antibodies at the time of the second (fig 1).

Serum alanine aminotransferase values were raised between 10 and 230 times (mean 46 times). Serum bilirubin concentrations were normal in four, raised in 17 (between 1·5 and 30 times, mean 10 times), and not measured in five. In 13 out of 18 instances where sufficient sera were available to permit evaluation in detail HBsAg titres were temporarily lowered at the onset of delta infection. In a

TABLE II—*Clinical features of delta infection in chronic carriers of HBsAg*

	Addicts	Non-addicts
Acute hepatitis (<2 months) ..	17	0
Prolonged hepatitis (>2 months) ..	8	0
Fulminant (fatal)	1	0
Subclinical hepatitis		2
Incomplete data	16	1
Total	42	3

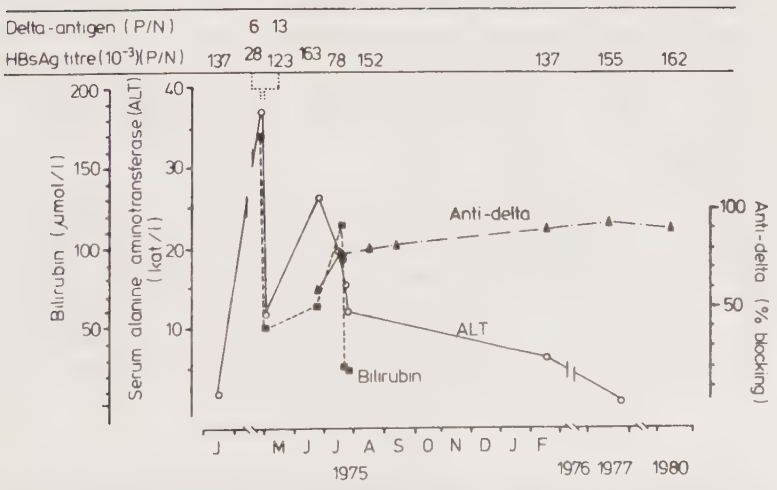

FIG 1—Acute hepatitis caused by the delta agent in a chronic carrier of HBsAg. Serum bilirubin concentrations and aminotransferase activity rose biphasically, delta-antigen was present at the first enzyme peak, and delta-antibody appeared at the time of the second peak.

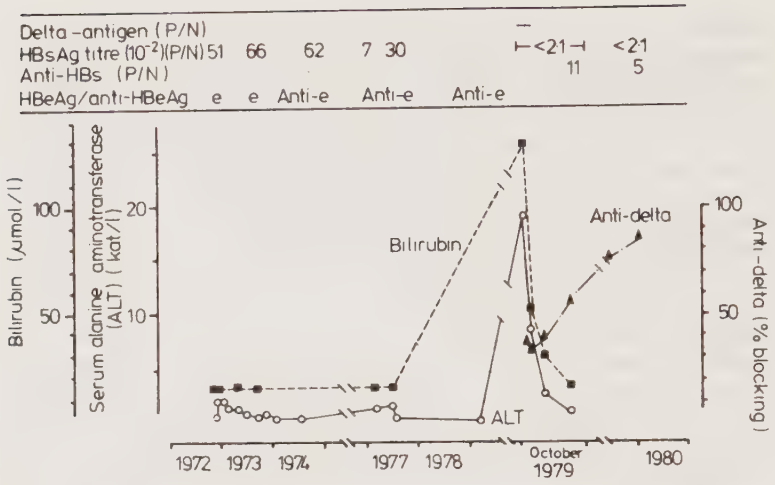

FIG 2—Acute hepatitis caused by the delta agent in a chronic HBsAg carrier after seven years of carriership. After aminotransferase activity and bilirubin concentrations returned to normal values HBsAg disappeared and anti-HBs developed. The anti-delta titre rose gradually during the following months.

further three patients the fall in titre persisted and anti-HBs developed. In two patients only was no fall in HBsAg titre observed. The change in titre occurring simultaneously with the first enzyme and bilirubin peak is shown in fig 1.

To establish whether delta infection is liable to occur at any particular time during the course of a chronic carrier state of HBsAg 25 carriers in whom the onset of both hepatitis B virus infection and delta infection were known were studied. This showed a wide spectrum from simultaneous onset to a time lag of seven years. In 26 patients the HBeAg/anti-HBe status was known at the time of onset of delta infection; eight of these were positive for HBeAg and 18 for anti-HBe. No conversion to anti-HBe during the delta infection was found except in one patient in whom a conversion to anti-HBs occurred simultaneously.

During follow-up of the 191 chronic carriers HBsAg disappeared in 19 after a carrier state lasting from eight months to 10 years (mean 38 months). When clearance occurred among delta-positive carriers the acute delta episode was directly related in time to the disappearance of HBsAg (followed by development of anti-HBs) in three patients. Fig 2 shows the disappearance of HBsAg during an acute episode of hepatitis occurring after seven years of carriership in a young man.

ACUTE HEPATITIS

Of 592 cases of acute hepatitis B, 57 were simultaneously infected with the delta agent, only one of whom was not a drug addict. Of

these, 40 had classical acute hepatitis which could not be distinguished from the other cases by either clinical signs or enzyme patterns. In eight a biphasic course was noted (fig 3); there was a double bilirubin and aminotransferase peak, and the delta antibody titre rose during the second peak. One further case had a prolonged course. In eight patients data were inadequate to permit evaluation.

A chronic HBsAg carrier state developed in two of those 57 patients who had simultaneous delta and hepatitis B virus infections, whereas

TABLE III—*No of chronic carriers of HBsAg in whom the antigen cleared during continuous follow-up*

	Antigen clearance in HBsAg carriers:			Total No of carriers
	Total No	Delta-positive	Delta-negative	
Addicts	14	9/42	5/37	79
Homosexual men	0		0/15	15
Haemophiliacs	0	0/1	0/7	8
Others (with known source of infection)	3		3/28	28
Unknown source of infection ..	2	0/2	2/59	61
Total	19	9/45	10/146	191

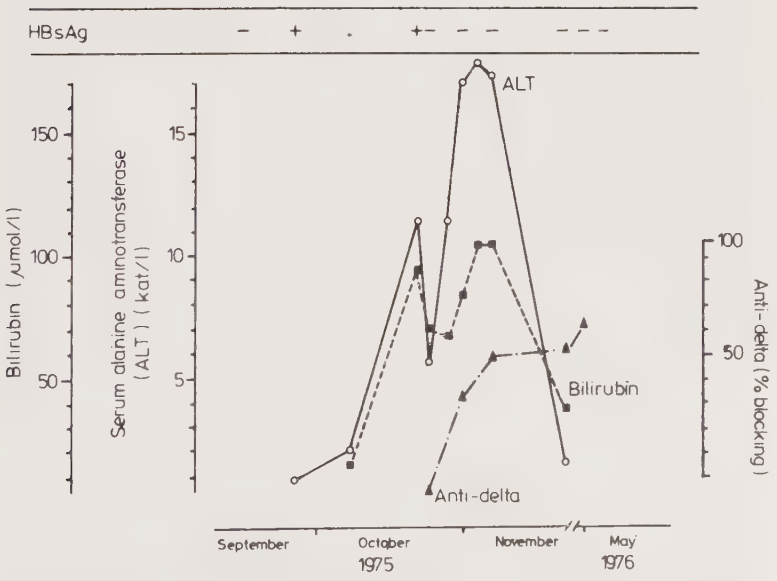

FIG 3—Simultaneous infection with hepatitis B virus and the delta agent causing a biphasic rise in serum bilirubin concentration and aminotransferase activity. Delta-antigen was not detected in this patient; the anti-delta titre rose during the second enzyme peak.

of 535 cases of hepatitis B without delta markers 25 became carriers (table IV). Thus, no difference in development of chronic hepatitis B virus infection could be seen between the two groups.

TABLE IV—*Clinical features of simultaneous acute infection with hepatitis B virus and the delta agent compared with type B hepatitis alone*

	No (%) delta-positive	No (%) delta-negative	Statistical significance
Classical acute self-limiting hepatitis ..	38 (66·6)	489 (91·4)	NS
Classical acute hepatitis progressing to chronic carrier state	2 (3·5)*	25 (4·7)	NS
Biphasic hepatitis	8 (14·1)	15 (2·8)	$p<0·001$
Prolonged acute hepatitis	1 (1·7)	1 (0·2)	NS
Subclinical hepatitis B	0	4 (0·7)	NS
Insufficient data	8 (14·1)	1 (0·2)	NS
	57 (100)	535 (100)	

*Also included among the 45 chronic carriers.

Discussion

Delta infection, as it occurs in our area, is overwhelmingly a phenomenon linked with intravenous drug addiction. Only four non-addicts harboured delta markers: a haemophiliac, a tourist who had received blood transfusions in Spain, and one Turkish and one West German immigrant.[4] The first instances of delta infection occurred in 1973 in two brothers (identical twins) who were both chronic HBsAg carriers and addicts and who were infected five months apart. In the same year the tourist referred to above was infected. From 1975 onwards there was a steady increase in the prevalence of delta markers, 72% of all addict carriers being positive for anti-delta by 1981.

Delta infection presumably spreads by the same routes as the hepatitis B virus, for example, by shared hypodermic needles. The present study also indicated potential infectivity of blood transfusions and coagulation-factor concentrates. Delta infection in a chronic carrier may occur at the beginning of the carrier state or at any time later as long as the carrier state persists.

The HBeAg/anti-HBe system has no bearing on the risk of an individual becoming infected with the delta agent. This accords with the concept that delta infection occurs in the presence of HBsAg whether the hepatitis B virus genome is integrated or not. The lower number of HBeAg-positive cases among delta-infected carriers reflects the natural history of the HBsAg carrier state in addicts, in whom a conversion from HBeAg to anti-HBe usually takes place after a comparatively

short time. Our results indicated that the likelihood of a case of acute hepatitis B infection developing into a chronic carrier state was not increased by additional infection with the delta agent. A similar conclusion has been reached by Smedile *et al.*[7]

The one example in our study of delta infection in a chronic carrier leading to fulminant hepatitis and death was unprecedented in our area. It was the only case of fulminant hepatitis B infection among 789 patients with either chronic carriership of HBsAg or acute-type hepatitis B infection registered between 1970 and 1981. An episode of hepatitic activity in a chronic drug addict and carrier should alert the clinician to the possibility of delta infection. In fact, several episodes previously classified by exclusion of hepatitis A as hepatitis non-A, non-B have now been shown by positive criteria to be instances of delta infection. A biphasic bilirubin and enzyme pattern also suggests delta hepatitis.

Delta infection occurring simultaneously with acute hepatitis B is not recognisable by clinical features alone. In a few instances an unusually protracted course may retrospectively suggest delta infection. Of greater relevance is a biphasic pattern with double rises in enzyme activity and bilirubin concentrations. These findings were also noted in animal experiments[3] and in patients in an Italian multicentre study.[6] In our study this pattern occurred with significantly higher frequency in the patients with both delta and hepatitis B virus infections (eight out of 44 cases) than among patients with hepatitis B virus infection alone (15 out of 535 cases) ($p < 0{\cdot}001$).

An interesting aspect of delta infection in chronic carriers is its connection with a depression in HBsAg titres. In the animal experiments by Rizzetto *et al*[3] a lowering of HBsAg titres was seen in both carrier chimpanzees infected. The titres, however, eventually reverted to preinfection values. In our patients this effect is shown in fig 1, where it was temporary, and in fig 2, where the HBsAg titre stayed permanently below the sensitivity threshold of the assay and anti-HBs developed. This finding has diagnostic and prognostic implications. As the HBsAg titre decreases it may temporarily become difficult to detect the antigen. A correct diagnosis of an HBsAg carrier may then be missed. Previously, when less sensitive assays were used, such diagnostic mistakes were particularly liable to occur.

Delta infection in a chronic carrier is sometimes associated with termination of the carrier state. In our series of 191 HBsAg carriers, 19 lost HBsAg over 12 years' follow-up. Fourteen of these were drug addicts, among whom nine had and five had not demonstrable markers of delta infection. Thus, antigen clearance not infrequently occurs irrespective of delta infection. In three cases, however, clearance coincided with an attack of acute

hepatitis and anti-HBs developed in all three cases, which suggests a causal relationship between delta infection and HBsAg clearance, a possibility which should be studied in more detail.

The discovery of the delta agent has improved our understanding of a number of obscure events in the natural history of hepatitis B virus infection in drug addicts. Thus, acute episodes of hepatitis, sometimes called hepatitis non-A, non-B, may actually be instances of delta infection. The clearance of HBsAg not infrequently seen in drug-addict carriers may also be associated with delta infection.

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Clinical and epidemiological aspects of chronic HBV infection in homosexual men and in drug addicts. A long-term follow-up.

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Abstract

A long-term follow-up of 95 chronic HBsAg carriers, 16 homosexual men and 79 intravenous addicts,disclosed fundamental differences between these two types of chronic HBsAg carriers.

There was a striking difference in the duration of viral replication as expressed by the presence of HBeAg. Two years after the initial contact with our department all homosexual men were still positive for the HBeAg whereas 77 per cent of the addicts had converted to anti-HBe.

Severe histological damage, i.e. chronic aggressive hepatitis, cirrhosis or primary liver cancer, was found in more than half of the homosexual men who underwent liver biopsy. In addicts negative for delta markers chronic persistent hepatitis was a regular finding. However, in addicts infected with the delta agent a similar degree of liver damage as in the homosexual men was found.

Introduction

After serological methods for the detection of Australia antigen had come into general use around 1970 it was learnt that although hepatitis B and inoculation hepatitis were used as synonymous terms many patients positive for the Australia antigen in fact had not received blood transfusions or taken illicit injections. Transmission in these patients appeared to be through sexual contact, homosexual as well as heterosexual (1-5). Thus arose the concept of hepatitis spread by non-parenteral or transmucosal routes. During the following decade a considerable amount of information about hepatitis B as a sexually transmitted disease has accumulated. It has been found particularly prevalent among the sexually promiscuous and modes of intercourse involving anal contact have been found important for the spread of hepatitis B (6). In several cross-sectional studies the prevalence of hepatitis B markers among homosexual men was found to be 50-60 per cent as against five per cent in the average population of North Western Europe and North America (7-9). Almost invariably this immunity has been acquired without overt signs of hepatitis and thus most hepatitis B events among homosexual men are subclinical. Mostly in connection with vaccine trials some longitudinal studies have defined the yearly incidence of hepatitis B in gay men to be between 13 and 28 per cent (8, 10, 11).

In this work we present a small but consecutive series of all homosexual hepatitis B surface antigen (HBsAg) carriers met with in a middle-sized Swedish city and followed during the period 1970-1984.

Intravenous addicts are well known to be at high risk of contracting hepatitis B. The study presents a long-term follow-up of a likewise consecutive series of addicts carrying HBsAg.

Together addicts and homosexual men form an important part of all chronic HBsAg carriers. The scope of the present work is to compare these two HBsAg-carrier populations in terms of clinical features, infectivity as expressed by the hepatitis B e antigen (HBeAg) or its antibody (anti-HBe), and histological liver damage.

Material

The patients derive from a consecutive series of acute and chronic hepatitis B seen mainly at the Department of Infectious Diseases at the General Hospital in Malmö (population 230 000), Sweden, 1970-1983. This is the only hospital in

the city which receives hepatitis patients except for the prison hospital where small numbers of patients are seen, sometimes referred from other parts of Sweden.

Of 759 cases of HBsAg positive hepatitis 340 occurred in drug addicts (250 males and 70 females) and 45 in homosexual men. Thirty-eight of the addicts and four of the homosexual men became chronic HBsAg carriers, i.e. remained positive for the HBsAg for more than six months. In addition 206 chronic carriers without a history of previous acute hepatitis were seen. Forty of these were addicts and twelve homosexuals. Thus the investigation deals with a total of 94 HBsAg carriers, namely 78 addicts (68 males, 10 females) followed for one to 13 years (mean 5.8 years) and 16 homosexual males followed for one to 9 years (mean 4.2 years). Age at first contact was 16-53 (mean 24.2) years in the addicts and 22-64 (mean 34.4) years in the homosexuals. These patients were initially seen in a variety of circumstances. As stated, one group sought medical attendance for signs of acute hepatitis. Others were seen for complaints of fatigue or vague abdominal discomfort without concomitant acute hepatitis. Some were discovered during evaluation of disease unrelated to hepatitis or as a result of routine screening in the departments of venereal disease, alcoholism, preventive medicine and dentistry, others were reported as possible sources of infection. All were advised to join a follow-up program with monthly, later on half-yearly, and yearly visits.

The purpose was, in equal measure, detection of chronicity, prevention of secondary cases by information to the patient and by prophylactic measures (particularly in the newborn) and, finally, protection of the patient himself (herself) against traumatic effects of sometimes quite exaggerated preventive measures taken by well-meaning but not always well-informed medical staff. The programme was well received but subsequent attendance was fully satisfactory only in the homosexual men. Indeed, the only occasion to observe some of the addicts arose during their repeated stays at the city jail.

Methods

Acute hepatitis was diagnosed on the basis of clinical symptoms such as jaundice, anorexia, nausea, upper abdominal pain, together with a more than ten-fold elevation of serum ALT. If patients were asymptomatic diagnosis was by laboratory criteria alone. Analysis for presence of type IgM antibodies to the hepatitis B core antigen (anti-HBc IgM) was carried out to confirm the diagnosis. A chronic carrier was defined as an individual positive for HBsAg

for more than six months.

Liver biopsies were carried out with a disposable needle (Hepafix^{R}) according to the technique of Menghini. The biopsies were with few exceptions read by the same pathologist without previous knowledge of clinical details. Biopsy criteria for chronic hepatitis were those of an international group (12).

HBsAg, anti-HBs, HBeAg and anti-HBe were determined by commercial radioimmunoassay kits (Abbot Laboratories, North Chicago, Ill., USA). Anti-HBc IgM for the detection of acute hepatitis B was determined by a solid-phase radioimmunoassay. (13). Delta-antigen and anti-delta were determined by a modification (14) of the original solid phase radioimmunoassay (15).

Results

A. Homosexual men

Clinical findings. At first presentation two patients had acute symptomatic hepatitis. Two more patients, although asymptomatic, had significant enzyme elevation (75-fold and 60-fold). In all four the diagnosis was confirmed by the detection of anti-HBc IgM.

In the remainder there were no clinical symptoms, anti-HBc IgM was not detected and the initial enzyme elevation was less than ten-fold (in seven) or unknown (five). During follow-up serum ALT generally remained elevated although a tendency towards lower levels and also occasional normal values were seen. Permanent normalization occurred only in one patient. No episode of acute hepatitis occurred during follow-up. Complaints of fatigue were common in the beginning, but in general the carriership caused little physical discomfort even in the presence of cirrhosis. One exception was the oldest patient who died from primary liver cancer at the age of 69. Extended sick-leave occurred in two instances. In one an ill-informed employer and the patient's working mates refused to have him come back for fear of infection, another patient experienced a neurotic-depressive reaction when his carriership was discovered. Spontaneous reactivation of chronic hepatitis B which has been described recently (16) in a group of chronic HBsAg carriers of whom many were homosexuals was not encountered.

Serological findings. As shown in table I all the patients were positive for HBeAg when first seen. Two years later the prevalence of HBeAg was still 100 per cent (13 patients), five years later it was 43 per cent (3/7). One patient

lost HBsAg after eight years of follow-up outside of the department at another clinic. He was the only patient to have received treatment with interferon. Markers of delta infection were not found in any patient in this group.

Histological findings. A total of 18 biopsies were carried out in 11 of the 16 patients. The results are presented in table II. It appears that in the five patients biopsied more than once the development was towards a more serious type of damage in four. Considering only the last biopsy the findings were: primary liver cancer (with cirrhosis and chronic aggressive hepatitis) in one, chronic aggressive hepatitis in five (with cirrhosis in two), chronic persistent hepatitis in three, chronic lobular hepatitis in one and acute unresolved hepatitis in one who was biopsied at the end of the first year of observation..

B.Addicts

Clinical findings. Thirty-eight chronic HBsAg carriers had acute hepatitis by clinical and/or laboratory criteria on first contact. Since they were HBsAg positive a diagnosis of acute hepatitis B was made. However, when these early sera were subsequently subjected to a series of serological assays that had not been available at the time of first contact the diagnosis turned out to be wrong in many patients.

Thus two patients were positive for IgM-type antibodies to the hepatitis A virus (anti-HAV IgM) and consequently had acute hepatitis A. Three had acute delta infection (in the absence of anti-HBc IgM this was delta superinfection rather than coinfection). One patient later found positive for anti-HBe in his first serum was diagnosed as non-A,non-B hepatitis (subsequently confirmed by absence of anti-HBc IgM) and one was belatedly diagnosed as acute alcoholic hepatitis. Of 19 patients in whom sera were available, twelve were positive for anti-HBc IgM (63 per cent). Two of these had acute delta coinfection. In 13 patients early sera were not available.

Among the 40 patients without a history of acute hepatitis three were positive for anti-HBc IgM in the first serum, indicating recent onset of infection.

During follow-up normal enzyme levels were noted on at least one occasion in 25 patients. In 16 it was the definite outcome of follow-up, in the remainder enzymes were again raised at the next visit. The role of hepatitis non-A,non-B infection could not be evaluated in these patients.

As in the homosexual group the HBsAg carriership as such caused little physical suffering. In contrast to the previous group, however, there were

several intervening episodes of acute hepatitis in the addicts. In 19 patients this was superinfection with the delta agent, frequently calling for extended periods of hospitalization, in four it was hepatitis A and in three presumably hepatitis non-A,non-B. One patient had acute hepatitis A and non-A,non-B, one and five years after spontaneous clearance of HBsAg and two patients had hepatitis A before becoming HBsAg carriers.

Six male patients died, one of fulminant superinfection with the delta agent, three others probably due to excessive doses of opiates, one died of intestinal bleeding and one was assassinated.

Serological findings. Fourteen (17.9 per cent) lost HBsAg after a mean observation period of five years (range 1-8 years). In three the clearance of antigen followed directly upon an acute infection with the delta agent. Anti-HBs developed in 11. There was no suggestion of HBsAg being lost after discontinuation of the drug habit.

As shown in table I 57 of the 78 patients (67 per cent) were HBeAg positive when first seen. After two years 23 per cent (14/61) and five years 8.1 per cent (3/37) were still HBe-antigen positive.

The fact that one third had already converted to anti-HBe when first seen indicates that many were established carriers already. The early conversion to anti-HBe seen in most of the addicts might therefore be an artifact. To test this possibility the period of HBe-antigen positivity until conversion was analyzed in those 15 patients in whom the onset of carriership could be dated by the presence of anti-core IgM in their first serum. Table I shows that these 15 patients were all HBe-antigen positive at the onset and that the conversion to anti-HBe occurred after similar intervals as in the total material.

Markers of delta infection were found in 63 per cent (41/71) of those carriers who were first seen after the introduction of the delta agent in 1973 (14).

Histological findings. Among addicts biopsied there is an overrepresentation of those who were symptomatic or had pronounced enzyme elevations (or both). As shown in table III a total of 27 biopsies were performed in 25 of the 78 carriers. In addition two post-mortems were carried out. As may be seen from the table primary liver cancer was absent and there was only one case of cirrhosis, in a 48 year old alcoholic dying from haematemesis and melaena. Seven had chronic aggressive and 14 chronic persistent hepatitis. Acute or reactive hepatitis was found in five who were biopsied after 2-5 years of

observation (mean 3.5 years).

In table IV addicts have been divided into those with and without markers of delta infection. The table comprises the histological findings into a more benign group (acute, reactive, chronic lobular and chronic persistent hepatitis) and a more severe group (chronic aggressive hepatitis, cirrhosis and primary liver cancer). More than half of the delta-positive group showed the severe picture, whereas the findings were benign in all of the delta-negative addicts. This difference is significant at the 0.01 level (chi square).

Comparison

The two groups were found to differ with regard to HBeAg-positivity (table I) and histological picture (table II-IV). Homosexual men were on the average ten years older than addicts at the time of presentation.

Statistical evaluation of the difference in HBe-antigen prevalence shows a significant difference at the 0.02, 0.001 and 0.05 level after one, two and three years respectively but not after four and five years.

Delta-infection is absent in our local homosexual community but widespread among addicts. As appears from table IV the degree of histological damage may be comparable in homosexuals and in delta-infected addicts, but it is of greater severity in homosexual men when compared to addicts without delta markers ($p < 0.01$, chi square).

Discussion

We have found that the natural history of chronic HBV infection is different in addicts and in homosexual men. Any report dealing with chronic HBsAg carriers should therefore clearly state the epidemiologic background of the patients and not draw general conclusions as to the overall natural history of chronic HBV infection, a concept which is largely an abstraction. A relevant example is a recent report (17) in which a series of chronic HBsAg carriers which to 77 per cent consisted of homosexual men was claimed to be representative of chronic HBsAg carriers in Britain as such. As pointed out in a subsequent letter (18) and as exemplified by our findings other groups of HBsAg carriers may have quite different characteristics. Besides, addicts constitute about 40 per cent of all HBsAg carriers in our city and homosexuals less than eight per cent.

We found the clinical course of the disease to be different in that most of the homosexual men were seen at the hospital at routine visits only, whereas many addicts, while neglecting routine visits, were nevertheless regular visitors due to a second, third and fourth attack of acute hepatitis at any time before, during, or, in the not infrequent event of spontaneous HBsAg clearance, after their HBsAg carriership. Multiple attacks of hepatitis in otherwise healthy addicts have been reported by others (19, 20). However, when such attacks occur in chronic HBsAg carriers there may be considerable confusion, particularly if the episode that brings the patient to attention for the first time is not hepatitis B. We found examples of such episodes caused by alcohol, by the agent(s) responsible for hepatitis non-A,non-B, by HAV and by the delta agent. It has been realized recently (21) that what causes a hitherto unrecognized HBsAg carrier to seek medical attention for the first time is not infrequently an acute superinfection with the delta agent. Our series contains three such patients belatedly recognized and two more with simultaneous HBV and delta infection. We did not routinely test for Epstein-Barr or cytomegalovirus infection since other Swedish workers (22) have shown these infections to be of little importance in clinical hepatitis.

The number of patients who were observed to have clinical or laboratory signs of acute hepatitis was four out of 16 (25 per cent) in the homosexual group. In the group of addicts there were difficulties in establishing the corresponding figure since early sera were not available (for analysis of anti-HBc IgM) in 13 patients. However, of 19 patients in whom analysis was possible, 12 (63 per cent) had anti-HBc IgM confirming the original diagnosis acute hepatitis B. If this percentage applies also to the 13 patients without early sera, eight of them must have had type B hepatitis at the outset. Thus a total of 20 addicts (26 per cent) had acute hepatitis at the beginning of their carriership, a similar figure as in the homosexual males.

The absence of delta infection in the homosexual group has recently been confirmed in nearby Copenhagen in an analysis of 260 homosexual men of whom 10 and 153 were positive for HBsAg and anti-HBs respectively (9).

HBeAg to anti-HBe conversion occurred much sooner in addicts than in homosexual men. A 50 per cent conversion was noted after one year in the former and after five years in the latter. The high prevalence of HBeAg and the low rate of conversion to anti-HBe in homosexual HBsAg carriers has been noted by earlier workers (17, 23, 24). It is unclear why continued viral replication for several years should be the rule in homosexual men but the exception in addicts. However, these differences may have important implications for the

two groups.

In an earlier epidemiological study (25) we have stated that in contrast to hepatitis A hepatitis B in addicts occurs continuously without an obvious epidemic pattern. This statement is correct when a comparison is made with the sharply delineated incidence peaks noted three times in a 10 year epidemiological curve of hepatitis A among addicts in our city. However, it is possible to discern high and low incidence periods even for hepatitis B and this pattern suggests a greater relative role of patients with acute disease than of chronic HBsAg carriers for transmission of hepatitis B. It is also fully consistent with the short period of infectivity found in addict HBsAg carriers. At any given time the number of contagious HBsAg carriers capable of spreading the disease is low among addicts.

Conversely, the yearly incidence of acute hepatitis B among homosexual males in our city is two to six cases without periodicity, which suggests a greater role for chronic HBsAg carriers in the spread of the disease. A pertinent point is the absence of overlapping between the two groups. There is no known or suspected example of intravenous addiction among any of the homosexual males in this investigation. An occasional male addict (not a hepatitis patient) has been known to offer his services as a prostitute to male homosexuals, but this has remained a marginal problem.

Liver biopsy was carried out in approximately one third of the addicts and in two thirds of the homosexual males.

In view of the lack of symptoms in the homosexual men the findings were unexpectedly serious as shown in table II. Six of 11 had either chronic aggressive hepatitis or cirrhosis or both. In one primary liver cancer developed. These results are comparable with a British report in which 45 per cent of a series of predominantly homosexual HBsAg carriers had either chronic aggressive hepatitis, cirrhosis or primary liver cancer (17). It was noteworthy that in five of our patients who underwent a second biopsy deterioration was noted in four.

In the addicts serious liver damage was found exclusively in patients with markers of delta infection. Of 14 patients eight had chronic aggressive hepatitis, one with cirrhosis. In contrast all the 13 delta negative patients showed potentially benign changes (table IV).

The serious prognosis of HBV infection with delta infection has been stressed by Italian researchers (26, 27). Only a small portion of patients in one of the Italian series was addicts, the majority representing the type of disease found in Southern Italy where delta infection is endemic. Our results indicate that

even among Scandinavian addicts chronic HBV infection, otherwise a relatively benign condition, may progress to serious liver damage once delta infection supervenes.

Higher age may be a factor of some importance since age has been shown to affect the prognosis in hepatitis adversely (29) but the age difference appears too small to account for the whole difference.

Table I

Persistence of HBeAg during follow-up in: A. Homosexual HBsAg carriers
B. Addict HBsAg carriers
C. Addict HBsAg carriers with defined onset of disease

Year of follow-up	A. Homosexual males		B. Addicts		C. Addicts with datable onset		p (A vs C)
	total number	number pos for HBeAg (per cent)	total number	number pos for HBeAg (per cent)	total number	number pos for HBeAg (per cent)	
0	16	16 (100)	78	52 (67)	15	15 (100)	
1	15	15 (100)	71	33 (47)	15	8 (53)	<0.02
2	14	14 (100)	61	14 (23)	13	3 (23)	<0.001
3	12	9 (75)	53	7 (13)	13	2 (15)	<0.05
4	8	6 (75)	48	5 (10)	11	2 (18)	N.S.
5	7	3 (43)	37	3 (8.1)	8	1 (13)	N.S.
6	4	2 (50)	27	2 (7.4)	4	1	
7	4	2 (50)	27	2 (7.4)	1	0	
8	3	1	22	2 (9.1)			
9	1	1	12	1 (8.3)			

Table II

Liver biopsy in 11 chronically HBsAg positive homosexual males.

		Year of follow-up								
	Year of birth	1	2	3	4	5	6	7	8	9
1	1946	CPH/CAH						CLH		
2	1954		CPH		CAH					
3	1913	AH		CAH Cirrh		PLC				
4	1949						CAH			
5	1931	AH					CPH			
6	1937	CAH				Cirrh				
7	1944		CAH	CAH Cirrh						
8	1943		CAH							
9	1956	AH								
10	1944				CPH					
11	1952		CPH							

Abbreviations:

AH	acute hepatitis
CAH	chronic aggressive (active) hepatitis
Cirrh	cirrhosis
CLH	chronic lobular hepatitis
CPH	chronic persistant hepatitis
PLC	primary liver cancer
React	Reactive hepatitis
UNS	Unspecific changes

Table III

Liver biopsy in 27 chronically HBsAg positive addicts

Nr	Year of birth	Sex	Year of follow-up 1	2	3	4	5	6	7	8	9	10	11
1	1951	M	CPH										
2	1950	M					AH*						
3	1951	M			CPH								
4	1951	M		CPH									
5	1950	M			AH								
6	1952	M	CPH										
7	1956	M		CPH									
8	1950	M	AH *								CPH		
9	1951	M		CPH									
10	1951	M	CPH										
11	1930	M					Cirrh						
12	1954	F	CPH										
13	1952	M								CPH			
14	1957	M		CPH									
15	1956	M	CPH										
16	1955	M		CAH									
17	1953	F				CAH							
18	1956	F		AH *									
19	1956	F	CPH				CAH						
20	1945	M		CAH									
21	1953	M					UNS						
22	1946	M						CPH					
23	1950	M				UNS							
24	1958	M	CAH										
25	1951	M	CAH										
26	1949	M	CPH										
27	1955	M											CAH

* acute delta infection

Abbreviations: see table II

Table IV

Comparison of liver biopsy findings in two groups of chronic carriers of HBsAg *

	Homosexual males	Intravenous addicts		
		with delta	without delta	total
Total	11	14	13	27
AH, React UNS	1	2	3	5
CLH	1	-	-	-
CPH	3	4	10	14
CAH	3	7	-	7
CAH + Cirrh	2	1	-	1
PLC	1	-	-	-

* The table includes the results of two post mortems.

Abbreviations: see table II

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HBV-DNA in the serum of patients with acute and chronic hepatitis B.

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Summary

Serial sera from 79 patients with acute self-limiting hepatitis, from 17 patients with acute hepatitis B evolving into a chronic HBsAg-carriership and from 43 chronic HBsAg-carriers without history of acute hepatitis were analyzed for presence of Hepatitis B virus-DNA by molecular hybridization technique.

In acute self-limiting hepatitis HBV-DNA was cleared within a few weeks of onset of clinical symptoms. The longest period of DNA-positivity observed in this group was 42 days.

When a chronic HBsAg-carriership developed HBV-DNA was present for no less than six months in all but one. Most chronic HBsAg-carriers eventually cleared HBV-DNA. Clearance frequently preceded conversion of HBeAg to anti-HBe and thus in many patients a transitional period with HBeAg but without HBV-DNA was found.

HBV-DNA is a more exact index of impending chronicity from HBeAg. A considerable overdiagnosis might result from using HBeAg as a marker of infectivity in acute as well as in chronic hepatitis. Infectivity in acute hepatitis B is a feature of the presymptomatic and early symptomatic phase.

Introduction

The diagnosis of hepatitis B has for the last 15 years been based on serological assays for the demonstration of hepatitis B surface antigen (HBsAg), the coat protein of the hepatitis B virus (HBV). However, most of the circulating HBsAg represents excess coat protein unassociated with complete, infectious virus particles. Therefore, additional assays are needed to determine whether complete virus particles are present. Hepatitis B "e" antigen has empirically been found to be associated with infectivity of chronic HBsAg carriers, while the corresponding antibody (anti-HBe) is a marker of low-degree infectivity. In many practical situations patients with anti-HBe are considered non-infectious although there are experimental (1, 2) as well as clinical (3-5) evidence of infectivity of HBsAg-positive patients with anti-HBe. Determination of DNA-polymerase, an enzyme contained in the hepatitis B virus (HBV) particle (the Dane particle) is a more direct measure of infectivity than HBeAg as is the demonstration of virus particles by electron microscopy. On the other hand, both of these methods are rather insensitive and laborious and relatively large volumes of sera are needed. Recently, sensitive and very specific nucleic acid hybridization techniques have been developed using radioactively labelled, cloned virus DNA as probes for the detection of viral nucleic acid in test specimens. We here present a longitudinal analysis of sera from patients with acute and chronic hepatitis B. Presence and subsequent clearance of HBV-DNA is demonstrated with a spot hybridization technique and compared with the HBeAg/anti-HBe system. The value of HBV-DNA as an index of infectivity and of impending chronicity is analyzed.

Material

1. From a total of 759 cases of acute hepatitis B seen at the Department of infectious diseases, General Hospital, Malmö. Sweden, 1972-1983, 79 patients were selected who had acute self-limiting hepatitis and in whom series of evenly spaced sera were available. The average number of HBsAg positive sera available for DNA analysis from each of the 79 patients was 6.8 (range 3-16).
2. From the same 759 patients were further selected 17 patients whose acute hepatitis evolved into a chronic HBsAg carriership.
3. From a group of approximately 200 chronic HBsAg carriers without a history of acute hepatitis were selected 43 patients who had been followed for an average of 58 months (range 11-120 months).

The composition of the material is shown in table I.

Methods

Acute hepatitis B was diagnosed on the basis of clinical symptoms (jaundice, anorexia, nausea, upper abdominal pain) together with a more than ten-fold elevation of alanine-aminotransferase (ALT) and temporary positivity of HBsAg. In the seventeen patients in whom acute hepatitis was followed by chronic HBs-antigenaemia the diagnosis of acute hepatitis B was confirmed by the detection of IgM antibodies to the Hepatitis B core antigen (anti-HBcIgM) in 14. In three, early sera were not available.

A chronic HBsAg carrier was defined as an individual positive for the HBsAg for more than six months.

Serological assays. HBsAg, anti-HBs, HBeAg and anti-HBe were determined by commercial radioimmunoassay kits (Abbott Laboratories, North Chicago, Ill., U.S.A.). Anti-HBc IgM was determined by a solid-phase radioimmunoassay (6). Delta antigen and anti-delta were determined by a modification (7) of the originally described solid-phase radioimmunoassay (8).

HBV DNA assay. A spot hybridization technique described by Scotto et al (9) for the detection of HBV DNA in serum was used with minor modifications. Fifty µl of each serum was transferred to the wells of a microtiter plate. To each well was further added 50 µl 0.14 M NaCl, 200 µl 1 M NaOH and 100 µl 2 M NaCl. The plate was incubated for 10 min at room temperature. By this step the Dane particles were ruptured and the DNA-denatured. With an eight-channel pipett the samples were transferred to a filtration apparatus (Schleicher & Schüll SRC-96) containing a nitrocellulose filter (Schleicher & Schüll BA 85) soaked with 6 x SSC (1 x SSC = 0.015 M Na_2-citrate, 0.15 M NaCl). The treated serum samples were filtered through the paper by vacuum. The nitrocellulose paper was then neutralised by filtering 200 µl 0.5 M Tris-HCl, 3 M NaCl (pH 7.4) through each well, air dried and baked at 80°C under vacuum for 2 hrs. Prehybridization was done over night at 42°C in 5 x SSPE (1 x SSPE = 0.18 M NaCl, 10 mM sodium-phosphate, 1 mM Na_2EDTA, pH 7.0), 5 x Denhardt's solution (10), 1 % glycine, 50 % formamide and 0.01 % sheared herring sperm DNA. Hybridization was performed over night at 42°C using 3.5 x 10^7 CPM per filter paper (96 samples) of cloned HBV DNA-pBR 322 (kindly provided by Dr Gerin), ^{32}P labelled by nick translation to specific activity 5 x 10^8 CPM per µg DNA. The hybridization solution con-

tained 5 x SSPE, 1x Denhardt's solution 0.01 % sheared herring sperm DNA, 0.3 % sodium dodecyl sulfate (SDS), 50 % formamide and the radiolabelled DNA probe. After the hybridization the paper was washed with 6x200 ml 10 mM phosphate buffer, 0.2 % SDS, 1 mM Na_2EDTA, pH 7 at 65°C for 2 hrs and autoradiographed at -70° for 3 days. The detection limit of the method was by testing dilution series of the cloned HBV DNA shown to be 1 - 10 pg DNA. However, the accuracy of this method has been questioned (9).

Results

The results are summarized in table II.

1. Acute self-limiting hepatitis.

HBV DNA was demonstrated in 56 of the 79 patients with acute hepatitis (71 %). There was no difference in the severity of the acute infection between DNA positive and negative patients as reflected by the highest enzyme value registered in each patient. There was a significant age difference, however, the average age of DNA positive and negative patients being 34.8 and 25.8 years, respectively. Table III shows the percentage DNA positive in the various age groups.

In 13 patients HBV DNA was found in one single early serum only. In seven it was positive for less than eight days, in 14 for 8-14 days, in seven for 15-21 days, in two for 22-28 days. In 10 patients DNA persisted for 30 days or longer. In three of these 10 patients the apparent long duration of DNA-positivity was a result of very early recognition of the patients. Two of them are presented in fig.s 1 and 2 demonstrating maximum levels of HBV-DNA well ahead of maximum enzyme elevation.

In two of the patients persistence of DNA for 33 and 48 days was associated with unusually protracted, biphasic types of hepatitis (negative for markers of delta-infection).

Only in five patients with apparently unremarkable acute hepatitis maximum periods of DNA-persistence (30, 34, 35, 39 and 42 days, respectively) were found.

There was no correlation between the patients' age and the duration of DNA-positivity.

HBeAg and HBV-DNA in acute hepatitis.

Of the 56 patients found positive for HBV-DNA 53 were also positive for HBeAg. One had anti-HBe and two were not analyzed.

Loss of HBeAg occurred before DNA-clearance (mean 14 days, range 4-37) in 12 patients. Average age in this group was 27.4 years (range 18-46). Simultaneous loss of HBeAg and HBV-DNA was seen in 11 patients. In 29 patients HBeAg remained positive after DNA-clearance for a mean of 22 days (3-96). The average age in this group was 37.6 years (17-84).

The extreme persistence of HBeAg for 96 days after HBV-DNA was observed in an 84 year old female with post transfusion hepatitis. The difference in age between patients loosing HBeAg before and after DNA-clearance is statistically significant ($p < 0.05$, student's t-test).

Of the 23 patients negative for DNA 18 were positive for HBeAg, seven in the first sample only. In the remaining 11 HBeAg was present for a mean of 32 days (13-111 days).

2. Acute hepatitis followed by chronic HBs-antigenaemia.

Of the 17 patients in this group four retained HBV-DNA throughout the observation period, i.e. for a mean of 32 months (range 9-54 months). They also remained HBeAg-positive.

In the remaining 13 patients clearance of DNA was observed after a mean of 23 months (range 2-57 months). With one exception all retained DNA for at least six months. The exception occurred in a 28 year old occasional drug user in whom HBV-DNA as well as HBeAg was cleared within 45 days of his first visit. Anti-HBcIgM was detected in the first blood sample.

The pattern of HBeAg to anti-HBe conversion among these patients was heterogenous, there were equal numbers of patients (four in each group) who lost HBeAg before, after and simultaneously with DNA-clearance. In the group where HBeAg was lost first it occurred 3 and 4 months before DNA-clearance, in two patients this interval could not be established. When HBeAg persisted it did so for 5, 5 and 12 months in three who subsequently were found negative. The fourth patient, however, was still HBeAg-positive 54 months after DNA-clearance was noted. Analysis of HBeAg/anti-HBe is still pending in one patient.

3. Chronic HBsAg carriers without a history of acute hepatitis.

HBV-DNA was demonstrated on at least one occation in 39 of 43 (91 %) of these patients who with one exception (case history I, fig 3) were all HBeAg-positive in their first serum.

In twelve patients DNA-clearance did not occur. Among these patients there were some with very long observation times (5, 5, 7, 8 and 9 years). The over all length of follow-up was 49 months (range 6-108 months). Eleven were positive for HBeAg throughout. The twelfth patient was positive for anti-HBe throughout (case history I, fig 3).

In 25 patients DNA-clearance was observed after a mean period of 15 months (3-72). HBeAg was initially found in all. It disappeared around the time of DNA-clearance in eight. Thus seventeen were still HBeAg-positive at the time of DNA-clearance and five remained so during the rest of the observation period, i.e. for a mean of 12 months (1-22 months) whereas 8 after additionally 8 months (3-22) of HBeAg eventually converted to anti-HBe. In four DNA-negative, HBeAg-positive patients further visits are scheduled.

In two patients with DNA in a single early serum the results of analysis of HBeAg/anti-HBe were inconclusive.

In four HBsAg-carriers (3 positive for HBeAg, 1 for anti-HBe) DNA was not found. Two of these were young Vietnamese immigrants aged 14 and 16 years, both positive for the HBeAg.

In two drug addicts the level of HBV-DNA could be studied during an acute superinfection with the delta-agent.

Case history I (fig 3)

This 25 year old male intravenous drug addict was discovered at a routine control to be HBsAg-positive. Being negative for anti-HBc IgM, positive for anti-HBe and having normal enzymes he was taken to have been a chronic HBsAg-carrier for some time already. After three months he developed acute delta superinfection with protracted enzyme elevation, six months later ALT was still increased by a factor 25 and biopsy showed chronic aggressive hepatitis. The patient was strongly positive for HBV-DNA in all 14 available sera. He was positive for anti-HBe in an early and in a late serum and negative for HBeAg

and anti-HBe in two sera obtained during the middle part of the observation period.

Case history II (fig 4)

Fig 4 shows delta infection in an HBeAg-positive HBsAg-carrier (female drug addict) occurring three years after HBsAg was first detected. During the acute delta episode, January - March 1979, there was a depression of HBsAg titers and HBV-DNA disappeared. It returned, however, after eight months and was finally cleared two years later.

Discussion

1. Acute hepatitis

HBV-DNA was demonstrated in 71 % of patients with acute self-limiting hepatitis. Since, presumably, viral DNA is present at some time in all patients infected with HBV an attempt should be made to explain its apparent absence in almost 30 % of the patients.

In case of late presentation of the patient DNA-clearance may already have been accomplished at the time of first contact. Such patients would have lower enzyme levels than patients presenting earlier during the disease. Indeed, in a few patients with low enzyme levels at the onset this may be the correct explanation why DNA was not found. However, on the average, the enzyme level was not lower in the DNA negative group and therefore late presentation accounts only for a small number of patients.

The causal agents of many viral diseases, e.g. hepatitis A are present mainly during the presymptomatic phase of the disease. This is probably the reason why viral DNA could not be found in 29 % of the patients. In two patients who for various reasons were encountered during the early incubation period (fig 1 and 2) the DNA-curve was already receding from peak level at a time when enzyme elevation had not yet reached its maximum. The interesting observation that DNA was almost invariably present in middle-aged and elderly but absent in half the patients younger than 20 suggests age-influence on DNA-clearance although there was no clearcut correlation between age and overall length of observed DNA-positivity.

When the times of DNA-clearance are compared with those of HBeAg loss a con-

siderable overlap is noticed. Absence of HBeAg in a patient positive for DNA is short-lived in most patients and not liable to create practical problems. On the other hand, retention of HBeAg after DNA-clearance was seen in more than half of all DNA-positive patients with HBeAg. It is generally accepted that HBV-DNA is a more specific indicator of infectivity than HBeAg. Our results demonstrate that a difference in the period of infectivity in the order of three weeks would result from relying on HBeAg compared to HBV-DNA. Three weeks more or less of infectivity period would mean much to many patients.

2. Acute hepatitis leading to chronic HBsAg-carriership.

If we compare the groups I and II we find that among the 79 patients with acute self-limiting hepatitis none was positive for HBV-DNA for more than 42 days whereas in those 17 patients in whom HBs antigenaemia became chronic DNA persisted for at least 6 months in all but one. This demonstrates the usefullness of HBV-DNA as an indicator of impending chronicity. The same forecast could had been made by means of HBeAg which was positive for more than six months in 15 (one patient had early disappearance of both DNA and HBeAg, in one results were inconclusive). However, among the patients with acute hepatitis eight were positive for HBeAg for 42-110 days and cleared HBsAg nevertheless. Therefore, of the two prognostic indices HBV-DNA seems to be the more reliable.

2. - 3. All chronic HBsAg carriers.

HBV-DNA in patients with chronic HBV-infection has recently become the subject of much study.

In the first work by Brechot et al (11) it was found that HBV-DNA and HBeAg in the serum together with free viral DNA in the liver are characteristic of one type of chronic HBsAg-carriers whereas integrated DNA in the liver and absence of DNA and HBeAg in serum are characteristic of another.

Bonino et al (12) related DNA in the serum of chronic HBsAg carriers to intrahepatic HBcAg and found 100 % correlation, whereas the correlation between intrahepatic HBcAg and serum-HBeAg was only 86 %. The authors suggested that infectivity of chronic HBsAg carriers with intrahepatic delta antigen in whom such markers as DNA-polymerase and HBeAg are often absent might be detected by the demonstration of DNA.

Weller et al (13) also detected HBV-DNA in occational patients negative for HBeAg and/or positive for anti-HBe.

Kam et al (14) described three groups of HBsAg carriers according to degree

of infectivity (high, low, absent). In the high infectivity group HBV-DNA and HBeAg as well as high titers of HBsAg are found in serum and in the liver free DNA predominates. In the low infectivity group anti-HBe but no viral DNA is found in serum, while in the liver both free and integrated DNA is present. In the third group without infectivity DNA is not demonstrable in the liver.

In subsequent works (15-17) the discussion has focused on HBsAg-carriers with chronic liver disease who although being positive for anti-HBe have been found infectious. It has been suggested that in the Mediterranean area such cases often are results of delta-infection (17).

We found a good correlation between viral DNA and HBeAg in those chronic HBsAg-carriers in whom DNA persisted throughout follow-up. There are 16 such patients, 15 were HBeAg-positive throughout.

In the 38 patients in whom DNA-clearance was seen (13 from group II and 25 from group III) the picture was less clearcut. Loss of HBeAg before DNA-clearance was only seen in four instances, it occurred three and four months before DNA-clearance in those two patients in whom sufficient sera were available. Thus, again the constellation HBeAg-negative/HBV-DNA-positive is not very important in our material. On the other hand, the combination HBeAg-positive/DNA-negative is common (17/39) and sometimes of long duration (8-12 months). There is one extreme example of a male HBsAg-carrier who was still HBeAg-positive at his most recent visit 54 months after clearance of DNA. In such patients HBeAg assumes similarities with the HBsAg in that there appears to be an excess production of both antigens unconnected with infective particles in the serum. Whatever the interpretation the clinical implications are obvious: if infectivity is defined according to the patients HBeAg/anti-HBe status a considerable overdiagnosis might result.

The risk of underdiagnosis, i.e. of missing infectivity in an hepatitis B-carrier positive for anti-HBe although not negligible, is probably not very great in our community. As stated by Sherlock and Thomas (18) for the UK and as shown here for Sweden anti-HBe-positive HBsAg-carriers are rarely HBV-DNA-positive. In Italy, on the contrary, a high percentage of such patients have HBV-DNA in serum and significant liver damage caused by delta-agent infection (17). Interestingly enough, the only example among our 60 HBsAg-carriers of HBV-DNA and anti-HBe coexisting in the same patient is a male drug-addict with chronic aggressive hepatitis due to delta-agent superinfection (fig 3).

The effect of delta superinfection on the level of HBV-DNA is shown in fig.s

3 and 4.

In fig 3 the patient is anti-HBe-positive, the level of HBV-DNA is unaffected and at maximum throughout.

In fig 4 the patient is HBeAg-positive, the effect of delta-superinfection on DNA is complete suppression with subsequent reappearance of DNA seven months later.

Whether these two cases represent typical patterns of the effect of delta superinfection remains to be established.

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Table I. Three groups of patients analysed for the presence of hepatitis B virus DNA.

	Total (male/ female)	Mean age (range)	Addicts	Homo-sexual males	Other known source of infection	Unknown source of infection
1. Acute self-limiting hepatitis B	79 (51/28)	32.4 (17-84)	30	9	13	27
2. Acute hepatitis B evolving into chronicity	17 (15/2)	28.2 (18-64)	11	3	-	3
3. Chronic HBsAg carriers without history of acute hepatitis	43 (36/7)	25.9 (0-64)	16	6	11*	10

* Two Swedish neonates and nine sino-vietnamese immigrants, all presumably infected at birth.

Table II. HBV-DNA and HBeAg in patients with acute and chronic hepatitis B.

	Total	HBeAg-pos
I. Acute hepatitis	79	71
HBV-DNA pos	56	53
HBV-DNA neg	23	18
II. Acute hepatitis evolving into chronic HBsAg carriership	17	16
HBV-DNA pos initially	17	16
clearance	13	12
no clearance	4	4
III. Chronic HBsAg carriership without history of acute hepatitis	43	41
HBV-DNA pos initially	39	38
clearance	25	25
no clearance	12	11
inconclusive	2	
HBV-DNA neg	4	3

Table III. HBV-DNA in the first serum drawn from each of 79 cases of acute, self-limiting hepatitis B with respect to the age of the patient.

Age years	Total	DNA pos (%)	DNA neg
< - 19	14	7 (50)	7
20 - 29	29	22 (76)	7
30 - 39	14	7 (50)	7
40 - 49	12	10 (83)	2
50 - 59	6	6 (100)	0
60 -	4	4 (100)	0

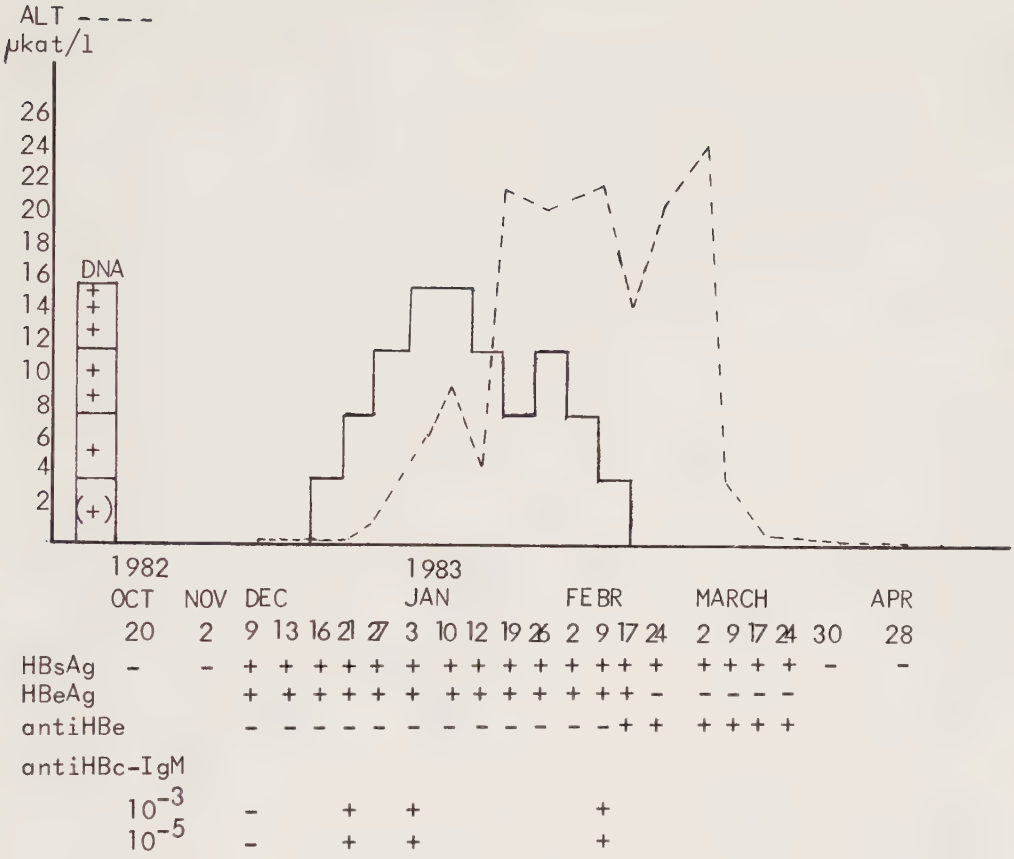

Fig. 1.

29 year old male, voluntary blood donor for several years, HBsAg negative on October 20 and November 2, 1982, but positive on December 9 with normal enzymes and without demonstrable DNA. The DNA appeared one week later and reached its maximum about one month later, well ahead of maximum ALT value. DNA was cleared three weeks before enzymes started to fall. HBsAg, however, dropped precipitously at the time of DNA clearance and conversion to anti-e occurred simultaneously.

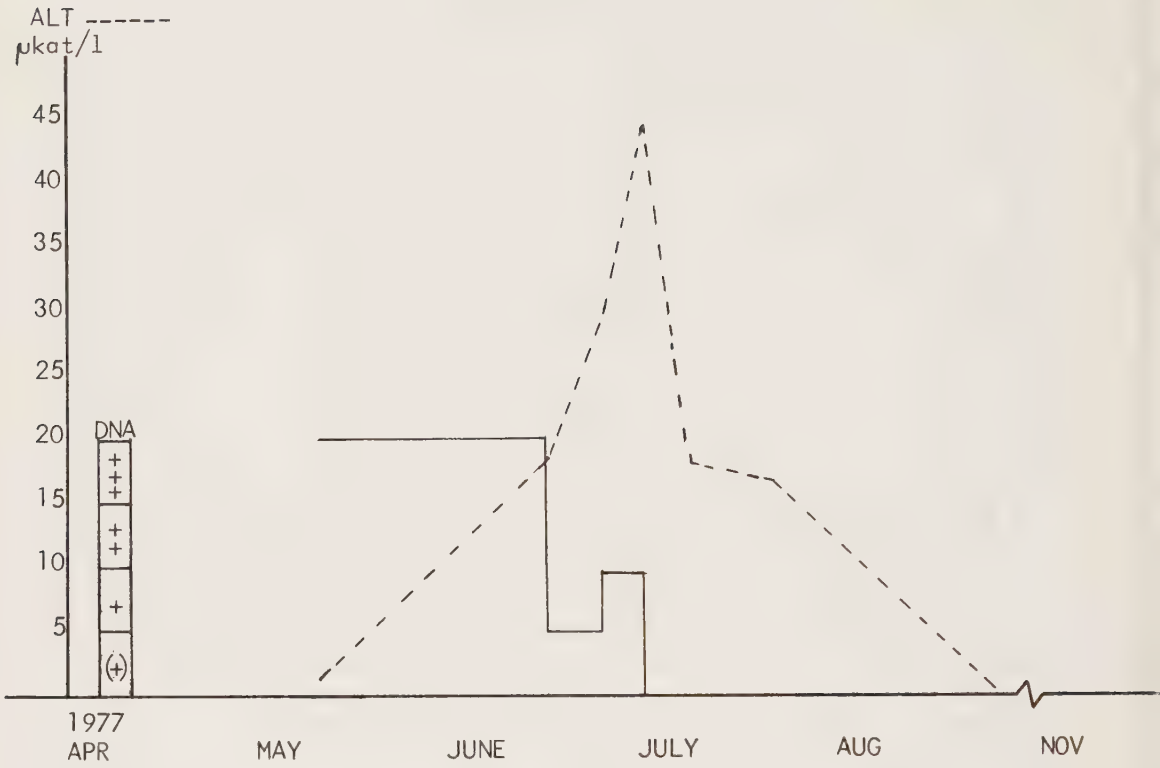

Fig. 2.

Twentyfour year old male blood donor and (since July 1976) amphetamine addict. Negative for HBsAg on April 25, 1977, but positive two weeks later when urticaria had developed. Jaundice appeared six weeks later when DNA was being cleared, maximum jaundice and ALT elevation occurred after DNA clearance.
The patient had simultaneous B and delta infection. Delta-antigen was found on July 19 and anti-delta one year later (not shown in figure).

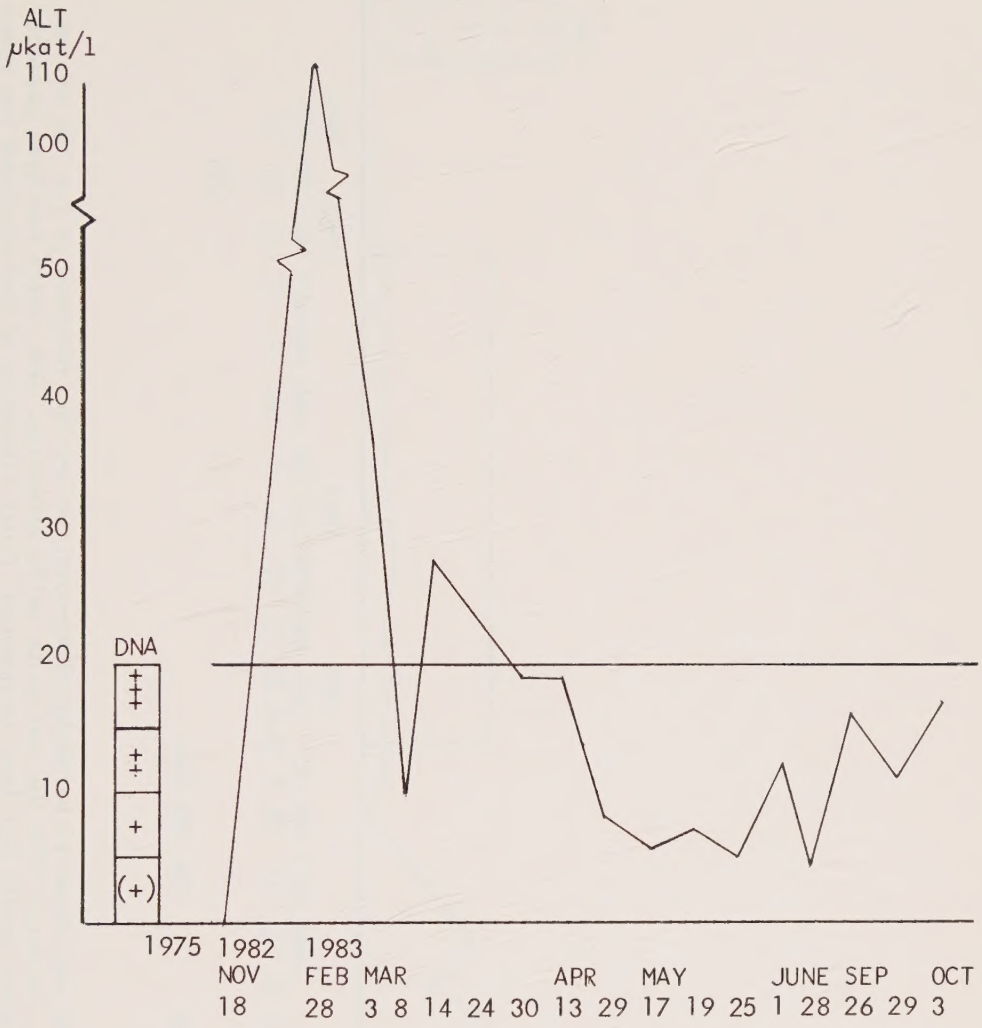

Fig. 3. Acute delta hepatitis in chronic HBsAg carrier positive for anti-HBe. Level of HBV DNA is unaffected throughout.

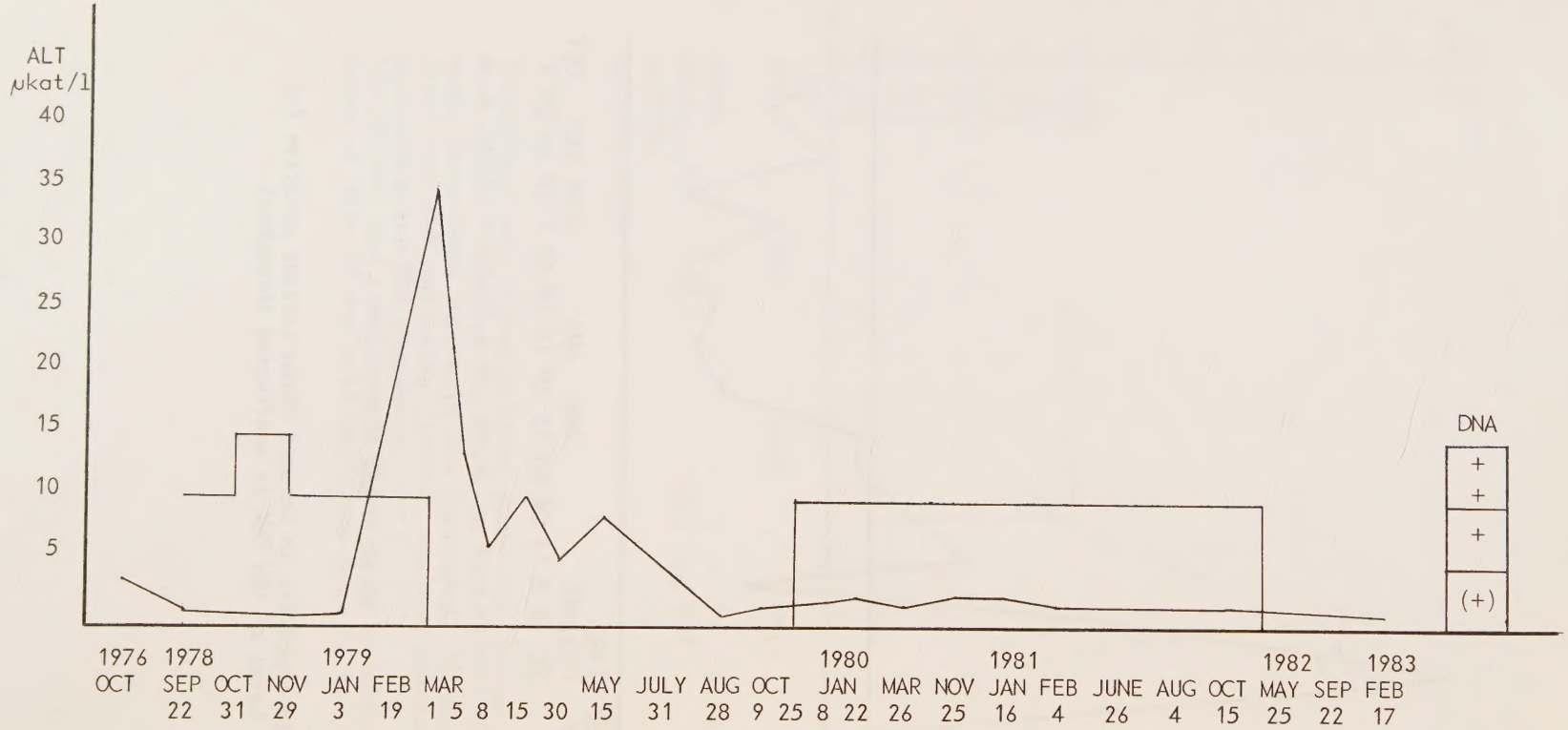

Fig. 4.
Case history
Twenty year old female addict who in 1976 was found to be HBeAg/HBsAg-positive and has remained so for the entire period of observation (now eight years). In March 1979 she had acute delta infection. DNA, which had been present for 2½ years, was not demonstrated for seven months but then reappeared. Final clearance of DNA occurred two years later.